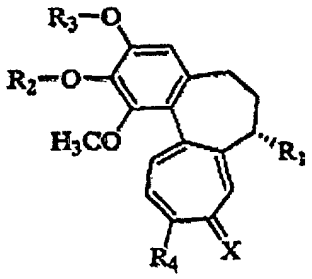
TRICYCLIC DERIVATIVES OR PHARMACEUTICALLY ACCEPTABLE SALTS THEREOF, THEIR PREPARATIONS AND PHARMACEUTICAL COMPOSITIONS CONTAINING THEM

FIELD OF THE INVENTION

The present invention relates to tricyclic derivatives represented by following <Formula 1>, or pharmaceutically acceptable salts thereof, their preparations and pharmaceutical compositions containing them.

<Formula 1>



(Wherein, R_1, R_2, R_3, R_4 and X are as defined in the description.)

BACKGROUND

One of pseudo-alkaloid compounds, colchicine has an anti-inflammation action, making it a therapeutic agent for rheumatoid arthritis [Internal Medicine, 86,

No.2, 342-345, 2000]. Colchicine and thiocolchicine derivatives have functions of muscle relaxation and anti-inflammation (USP 5 973 204, EP 0870761 A1). Thiocolchicoside has been used for the treatment of contracture and inflammation in skeletal muscles. Also, colchicine inhibits infiltration of monocytes and T-cells in a transplanted organ in animal experiment and at the same time restrains the production of TNF-α, IL-1 and IL-6, inflammatory cytokines, suggesting an inhibiting effect on immune response [*J. Am. Soc. Nephrol.*, 4(6), 1294-1299, 1993; *Transplantation Proceedings*, 32, 2091-2092, 2002]. Thus, colchicine is very attractive candidate for the development of an immune response inhibitor (WO 02/100824).

Colchicine inhibits a microtubule assembly by the interaction with tubulin, resulting in the suppression of cell division [*The Alkaloids*, 1991, 41, 125-176; USP 4 533 675]. Such colchicine has been used for the treatment of gout and other inflammatory diseases related to gout. However, the use of colchicine is limited to an acute inflammatory disease because of the limitation in therapeutic index and toxicity to gastrointestinal tract [*Pharmacotherapy*, 11, 3, 196-211, 1991].

All the endeavors to develop colchicine derivatives as an anticancer drug have not been successful so far [USP 3 222 253; USP 00/6080739; WO 97/01570], and only demecolcine has been used for the treatment of leukemia. However, toxicity to gastrointestinal tract and limitation in therapeutic index are still problems of demecolcine.

The present inventors have completed this invention by developing colchicine derivatives having excellent activities of anticancer, anti-proliferation and angiogenesis inhibition that have now stable therapeutic index resulted from decreased toxicity.

SUMMARY OF THE INVENTION

It is an object of the present invention to provide tricyclic derivatives or pharmaceutically acceptable salts thereof having excellent activities of anti-cancer, anti-proliferation and angiogenesis inhibition with stable therapeutic index by reduced toxicity.

It is also an object of this invention to provide a preparation method for tricyclic derivatives or pharmaceutically acceptable salts thereof.

It is a further object of this invention to provide a pharmaceutical composition containing tricyclic derivatives or pharmaceutically acceptable salts thereof as an effective ingredient.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a graph showing the changes of the volume of a tumor in a BALB/c nude mouse transplanted with human lung cancer cell line NCI-H460 after the administration of tricyclic derivatives of the present invention (Example 8),

FIG. 2 is a graph showing the changes of the body weight of a BALB/c nude mouse transplanted with human lung cancer cell line NCI-H460 after the administration of tricyclic derivatives of the present invention (Example 8),

FIG. 3 is a graph showing the changes of the volume of a tumor in a BALB/c nude mouse transplanted with human lung cancer cell line NCI-H460 after the

administration of tricyclic derivatives of the present invention (Example 12) by different concentrations (1, 3, 10 mg/kg),

FIG. 4 is a graph showing the changes of the body weight of a BALB/c nude mouse transplanted with human lung cancer cell line NCI-H460 after the administration of tricyclic derivatives of the present invention (Example 12) by different concentrations (1, 3, 10 mg/kg),

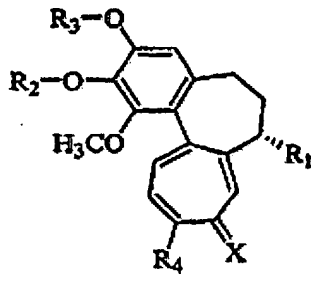
FIG. 5 is a set of photographs showing the volume of a tumor growing in a BALB/c nude mouse transplanted with human lung cancer cell line NCI-H460, which was separated on the 14^{th} day after the administration of tricyclic derivatives of the present invention,

FIG. 6 is a set of photographs showing the activity of tricyclic derivatives of the present invention to inhibit angiogenesis in HUVEC cells.

DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS

The present invention relates to tricyclic derivatives represented by following <Formula 1>, or pharmaceutically acceptable salts thereof.

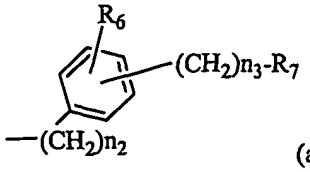
<Formula 1>

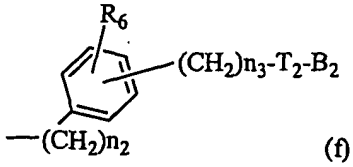


(Wherein,

(1) R_1 is $-T_1-B_1$;

in which T_1 is $-X_1-$, $-X_1-C(X_2)-$, $-N(R_5)-$, $-N(R_5)C(X_2)-$, $-N(R_5)S(O)n_1-$, $-N(R_5)C(O)-X_1-$ or $-N(R_5)C(X_1)NH-$, in that X_1 and X_2 are each O or S, R_5 is each H or C_1~C_5 alkyl group, n_1 is an integer of 1~2; and B_1 is selected from a group consisting of following (a)~(j),


(a)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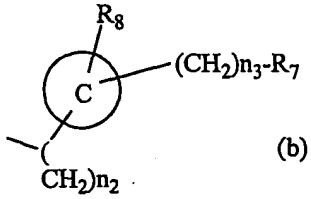
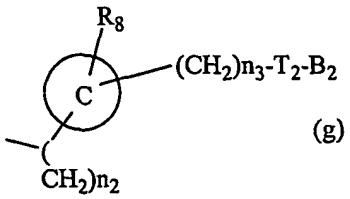

(b)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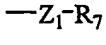
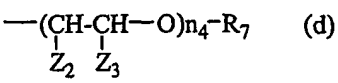
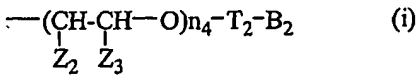
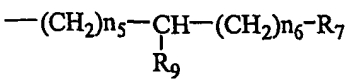

(c)

(h)


(d)


(i)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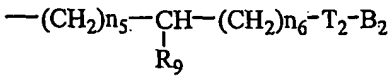
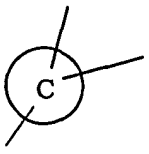
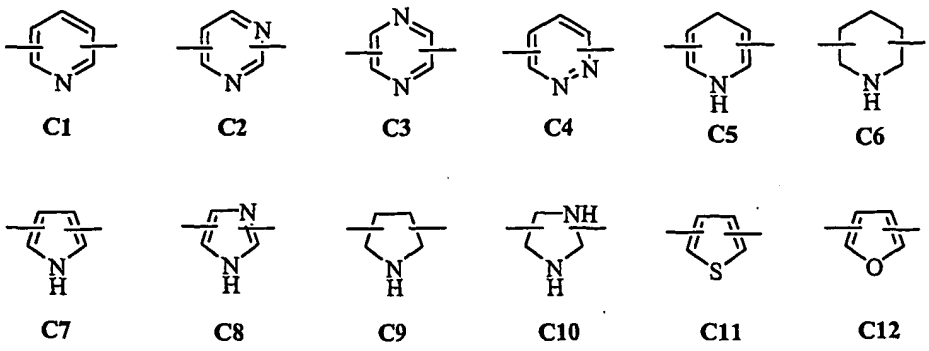

(j)

Wherein, R_6 and R_8 are each H, halogen, hydroxy, C_1~C_3 alkoxy, amino, nitro, cyano or C_1~C_3 lower alkyl group; R_7 and R_9 are each independently halogen, hydroxy, mercapto, -ONO, $-ONO_2$ or SNO, in which R_7 and R_9 are same or different;


is C_5~C_6 membered saturated or unsaturated heterocyclic ring containing 1~2 of hetero atom, in which the hetero atom is selected from a group consisting of O, S and N, preferably,



more preferably, C1 (pyridyl group) substituted at position 2 and 6 or position 2 and 5, C7 (pyrrolyl group) substituted at position 2 and 5 or position 2 and 4, C11 (thiophenyl group) or C12 (furanyl group); Z_1 is C_1~C_{10} straight-chain or branched-chain alkyl group, preferably C_2~C_5 straight-chain or branched-chain alkyl group or cycloalkyl group having substituent; Z_2 and Z_3 are each independently H or methyl group, in which Z_3 is H when Z_2 is methyl group, Z_2 is H when Z_3 is methyl group; T_2 is $-X_1-$ or $-X_1-C(X_2)-$, in that X_1 and X_2 are each independently O or S; B_2 is selected from a group consisting of said (a), (b), (c), (d) or (e); n_2 is an integer of 0~3, n_3 is an integer of 0~5, n_4 is an integer of 1~5, n_5 and n_6 are each independently an integer of 1~6;

(2) R_2 and R_3 are each independently H, $-PO_3H_2$, phosphonate, sulfate, C_3~C_7 cycloalkyl, C_2~C_7 alkenyl, C_2~C_7 alkynyl, C_1~C_7 alkanoyl, C_1~C_7 straight-chain or branched-chain alkyl or sugar, in which sugar is a

monosaccharide such as glucuronyl, glucosyl or galactosyl;

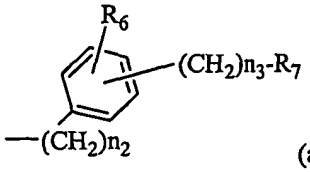
(3) R_4 is OCH_3, SCH_3 or $NR_{10}R_{11}$, in which R_{10} and R_{11} are each independently H or C_{1-5} alkyl;

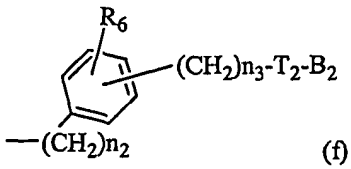
(4) X is O or S.)

Preferably in the compound of <Formula 1>,

(1) R_1 is $-T_1-B_1$;

in which T_1 is $-N(R_5)C(X_2)-$, $-N(R_5)C(O)-X_1-$ or $-N(R_5)C(X_1)NH-$, in that X_1 and X_2 are each O, R_5 is each H or C_1~C_5 alkyl group; and B_1 is selected from a group consisting of following (a)~(j),


(a)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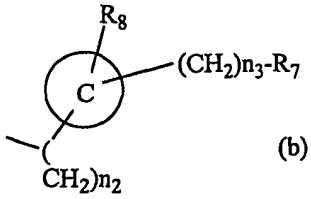
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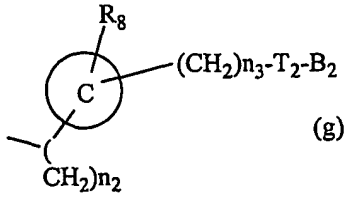
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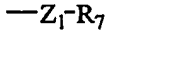
(c)

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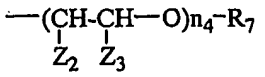
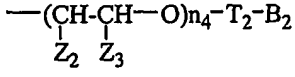
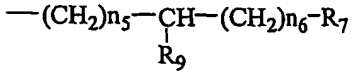
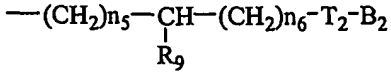
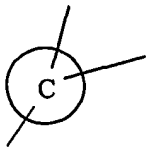

(d)


(i)

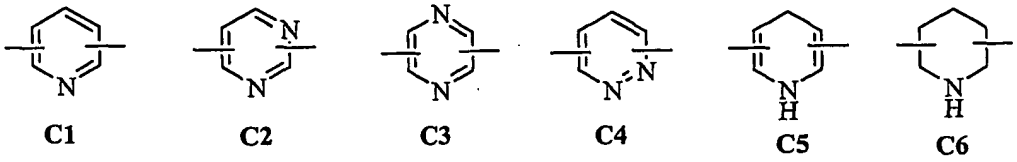

(e)


(j)

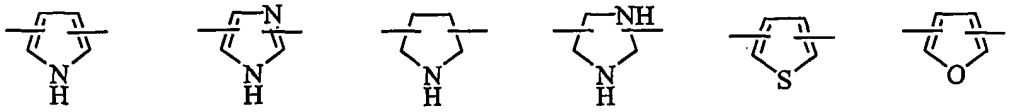
Wherein, R_6 and R_8 are each H, halogen, hydroxy, $C_1 \sim C_3$ alkoxy, amino, nitro, cyano or $C_1 \sim C_3$ lower alkyl group; R_7 and R_9 are each independently halogen, hydroxy, mercapto(thiol), -ONO, $-ONO_2$ or SNO, in which R_7 and R_9 are same or different;



is $C_5 \sim C_6$ membered saturated or unsaturated heterocyclic ring containing 1~2 of hetero atom, in which the hetero atom is selected from a group consisting of O, S and N, preferably,



C1 C2 C3 C4 C5 C6



C7 C8 C9 C10 C11 C12 ,

more preferably, C1 (pyridyl group) substituted at position 2 and 6 or position 2 and 5, C7 (pyrrolyl group) substituted at position 2 and 5 or position 2 and 4, C11 (thiophenyl group) or C12 (furanyl group), a bond of substituents may be at symmetrical or asymmetrical position; Z_1 is $C_1 \sim C_{10}$ straight-chain or branched-chain alkyl group, preferably $C_2 \sim C_5$ straight-chain or branched-chain alkyl group or cycloalkyl group

having substituent; Z_2 and Z_3 are each independently H or methyl group, in which Z_3 is H when Z_2 is methyl group, Z_2 is H when Z_3 is methyl group; T_2 is $-X_1-$ or $-X_1-C(X_2)-$, in that X_1 and X_2 are each O or S; B_2 is selected from a group consisting of said (a), (b), (c), (d) or (e); n_2 is an integer of 0~3, n_3 is an integer of 0~5, n_4 is an integer of 1~3, n_5 and n_6 are each independently an integer of 1~3;

(2) R_2 and R_3 are each independently C_3~C_7 cycloalkyl or C_1~C_7 alkyl;

(3) R_4 is SCH_3 or OCH_3;

(4) X is O or S.

Preferably, the compounds of <Formula 1> comprise:

1) 6-nitrooxymethyl-N-[(7S)-1,2,3-trimethoxy-10-methylsulfanyl-9-oxo-5,6,7,9-tetrahydro-benzo[a]heptalen -7-yl]-nicotineamide;

2) 5-nitrooxymethyl-furan-2-carboxylic acid-[(7S)-1,2,3-trimethoxy-10-methylsulfanyl-9-oxo-5,6,7,9-tetrahydro-benzo[a]heptalen-7-yl]-amide;

3) N-[(7S)-3-isopropoxy-1,2-dimethoxy-10-methyl-sulfanyl-9-oxo-5,6,7,9-tetrahydro-benzo[a]heptalen-7-yl]-3-nitrooxymethyl-benzamide;

4) N-[(7S)-3-ethoxy-1,2-dimethoxy-10-methylsulfanyl-9-oxo-5,6,7,9-tetrahydro-benzo[a]heptalen-7-yl]-3-nitrooxymethyl-benzamide;

5) 6-nitrooxymethyl-pyridine-2-carboxylic acid-[(7S)-1,2,3-trimethoxy-10-methylsulfanyl-9-oxo-5,6,7,9-tetrahydro-benzo[a]heptalen-7-yl]-amide;

6) 5-nitrooxymethyl-thiophene-2-carboxylic acid-[(7S)-1,2,3-trimethoxy-10-methylsulfanyl-9-oxo-5,6,7,9-tetrahydro-benzo[a]heptalen-7-yl]-amide;

7) N-[(7S)-3-cyclopentyloxy-1,2-dimethoxy-10-methylsulfanyl-9-oxo-5,6,7,9-tetrahydro-benzo[a]heptalen -7-yl]-3-nitrooxymethyl-benzamide;

8) N-[(7S)-3-ethoxy-1,2-dimethoxy-10-methylsulfanyl-9-oxo-5,6,7,9-tetrahydro-benzo[a]heptalen-7-yl]-2-fluoro-3-nitrooxymethyl-benzamide;

9) 2-fluoro-N-[(7S)-3-isopropoxy-1,2-dimethoxy-10-methylsulfanyl-9-oxo-5,6,7,9-tetrahydro-benzo[a]heptalen-7-yl]-3-nitrooxymethyl-benzamide;

10) 2-fluoro-3-nitrooxymethyl-N-[(7S)-1,2,3-trimethoxy-10-methylsulfanyl-9-oxo-5,6,7,9-tetrahydro-benzo[a]heptalen-7-yl]-benzamide;

11) N-[(7S)-3-cyclopentyloxy-1,2-dimethoxy-10-methylsulfanyl-9-oxo-5,6,7,9-tetrahydro-

benzo[a]heptalen-7-yl]-2-fluoro-3-nitrooxymethyl-benzamide;

12) 3-fluoro-5-nitrooxymethyl-N-[(7S)-1,2,3-trimethoxy-10-methylsulfanyl-9-oxo-5,6,7,9-tetrahydro-benzo[a]heptalen-7-yl]-benzamide;

13) N-[(7S)-3-ethoxy-1,2-dimethoxy-10-methylsulfanyl-9-oxo-5,6,7,9-tetrahydro-benzo[a]heptalen-7-yl]-3-fluoro-5-nitrooxymethyl-benzamide;

14) 3-fluoro-N-[(7S)-3-isopropoxy-1,2-dimethoxy-10-methylsulfanyl-9-oxo-5,6,7,9-tetrahydro-benzo[a]heptalen-7-yl]-5-nitrooxymethyl-benzamide;

15) N-[(7S)-3-cyclopentyloxy-1,2-dimethoxy-10-methylsulfanyl-9-oxo-5,6,7,9-tetrahydro-benzo[a]heptalen-7-yl]-3-fluoro-5-nitrooxymethyl-benzamide;

16) 4-fluoro-3-nitrooxymethyl-N-[(7S)-1,2,3-trimethoxy-10-methylsulfanyl-9-oxo-5,6,7,9-tetrahydro-benzo[a]heptalen-7-yl]-benzamide;

17) 2-fluoro-5-nitrooxymethyl-N-[(7S)-1,2,3-trimethoxy-10-methylsulfanyl-9-oxo-5,6,7,9-tetrahydro-benzo[a]heptalen-7-yl]-benzamide;

18) 3-hydroxy-5-nitrooxymethyl-N-[(7S)-1,2,3-trimethoxy-10-methylsulfanyl-9-oxo-5,6,7,9-tetrahydro-benzo[a]heptalen-7-yl]-benzamide;

19) 3,5-*bis*-nitrooxymethyl-N-[(7S)-1,2,3-trimethoxy-10-methylsulfanyl-9-oxo-5,6,7,9-tetrahydro-benzo[a]heptalen-7-yl]-benzamide;

20) 2-hydroxy-4-nitrooxymethyl-N-[(7S)-1,2,3-trimethoxy-10-methylsulfanyl-9-oxo-5,6,7,9-tetrahydro-benzo[a]heptalen-7-yl]-benzamide;

21) 4-nitrooxymethyl-thiophene-2-carboxylic acid [(7S)-1,2,3-trimethoxy-10-methylsulfanyl-9-oxo-5,6,7,9-tetrahydro-benzo[a]heptalen-7-yl]-amide;

22) 3-nitrooxymethyl-thiophene-2-carboxylic acid [(7S)-1,2,3-trimethoxy-10-methylsulfanyl-9-oxo-5,6,7,9-tetrahydro-benzo[a]heptalen-7-yl]-amide;

23) 2-(3-nitrooxymethyl-phenyl)-N-[(7S)-1,2,3-trimethoxy-10-methylsulfanyl-9-oxo-5,6,7,9-tetrahydro-benzo[a]heptalen-7-yl]-acetamide;

24) 3-(2-nitrooxy-ethyl)-N-[(7S)-1,2,3-trimethoxy-10-methylsulfanyl-9-oxo-5,6,7,9-tetrahydro-benzo[a]heptalen-7-yl]-benzamide;

25) 3-nitrooxybenzoic acid-5-[(7S)-1,2,3-trimethoxy-10-methylsulfanyl-9-oxo-5,6,7,9-tetrahydro-benzo[a]heptalen-7-yl-carbamoyl]-pyridine-2-yl-methylester;

26) 4-nitrooxybutyric acid-5-[(7S)-1,2,3-trimethoxy-10-methylsulfanyl-9-oxo-5,6,7,9-tetrahydro-

benzo[a]heptalen-7-yl-carbamoyl]-pyridine-2-yl-methylester;

27) 3-nitrooxymethyl-benzoic acid-6-[(7S)-1,2,3-trimethoxy-10-methylsulfanyl-9-oxo-5,6,7,9-tetrahydro-benzo[a]heptalen-7-yl-carbamoyl]-pyridine-2-yl-methylester;

28) 4-nitrooxybutyric acid-6-[(7S)-1,2,3-trimethoxy-10-methylsulfanyl-9-oxo-5,6,7,9-tetrahydro-benzo[a]heptalen-7-yl-carbamoyl]-pyridine-2-yl-methylester;

29) 3-nitrooxymethyl-benzoic acid-2-[(7S)-1,2,3-trimethoxy-10-methylsulfanyl-9-oxo-5,6,7,9-tetrahydro-benzo[a]heptalen-7-yl-carbamoyl]-phenylester;

30) 4-nitrooxybutyric acid-2-[(7S)-1,2,3-trimethoxy-10-methylsulfanyl-9-oxo-5,6,7,9-tetrahydro-benzo[a]heptalen-7-yl-carbamoyl]-phenylester;

31) 3-nitrooxymethyl-benzoic acid-3-[(7S)-1,2,3-trimethoxy-10-methylsulfanyl-9-oxo-5,6,7,9-tetrahydro-benzo[a]heptalen-7-yl-carbamoyl]-phenylester;

32) 4-nitrooxybutyric acid-3-[(7S)-1,2,3-trimethoxy-10-methylsulfanyl-9-oxo-5,6,7,9-tetrahydro-benzo[a]heptalen-7-yl-carbamoyl]-phenylester;

33) 3-nitrooxymethyl-benzoic acid-3-[(7S)-1,2,3-trimethoxy-10-methylsulfanyl-9-oxo-5,6,7,9-tetrahydro-benzo[a]heptalen-7-yl-carbamoyl]-benzylester;

34) 4-nitrooxybutyric acid-3-[(7S)-1,2,3-trimethoxy-10-methylsulfanyl-9-oxo-5,6,7,9-tetrahydro-benzo[a]heptalen-7-yl-carbamoyl]-benzylester;

35) 2-nitrosothio-N-[(7S)-1,2,3-trimethoxy-10-methylsulfanyl-9-oxo-5,6,7,9-tetrahydro-benzo[a]heptalen-7-yl]-benzamide;

36) 3-nitrosooxymethyl-N-[(7S)-1,2,3-trimethoxy-10-methylsulfanyl-9-oxo-5,6,7,9-tetrahydro-benzo[a]heptalen-7-yl]-benzamide;

37) 3-fluoro-5-nitrosooxymethyl-N-[(7S)-1,2,3-trimethoxy-10-methylsulfanyl-9-oxo-5,6,7,9-tetrahydro-benzo[a]heptalen-7-yl]-benzamide;

38) 3-nitrosothiomethyl-N-[(7S)-1,2,3-trimethoxy-10-methylsulfanyl-9-oxo-5,6,7,9-tetrahydro-benzo[a]heptalen-7-yl]-benzamide;

39) 3-fluoro-5-nitrosothiomethyl-N-[(7S)-1,2,3-trimethoxy-10-methylsulfanyl-9-oxo-5,6,7,9-tetrahydro-benzo[a]heptalen-7-yl]-benzamide;

40) 3-fluoro-5-nitrooxymethyl-N-[(7S)-1,2,3,10-tetramethoxy-9-oxo-5,6,7,9-tetrahydro-benzo[a]heptalen-7-yl]-benzamide;

41) 3-nitrooxymethyl-N-methyl-N-[(7S)-1,2,3-trimethoxy-10-methylsulfanyl-9-oxo-5,6,7,9-tetrahydro-benzo[a]heptalen-7-yl]-benzamide;

42) 3-fluoro-N-methyl-5-nitrooxymethyl-N-[(7S)-1,2,3-trimethoxy-10-methylsulfanyl-9-oxo-5,6,7,9-tetrahydro-benzo[a]heptalen-7-yl]-benzamide;

43) 2-(3-fluoro-5-nitrooxymethyl-phenyl)-N-[(7S)-1,2,3-trimethoxy-10-methylsulfanyl-9-oxo-5,6,7,9-tetrahydro-benzo[a]heptalen-7-yl]-acetamide; or

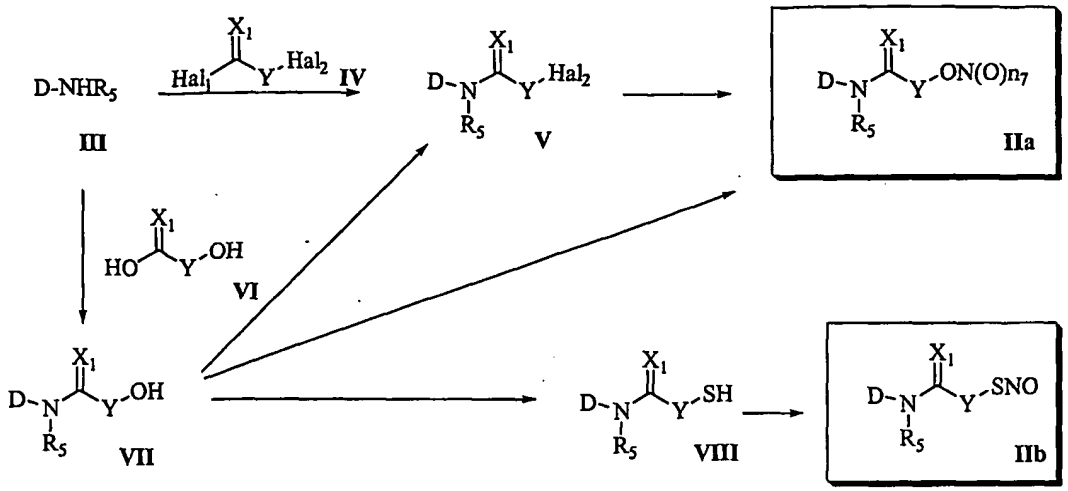
44) 2-(2-fluoro-5-nitrooxymethyl-phenyl)-N-[(7S)-1,2,3-trimethoxy-10-methylsulfanyl-9-oxo-5,6,7,9-tetrahydro-benzo[a]heptalen-7-yl]-acetamide.

The present invention also provides pharmaceutically acceptable salts of the compound represented by <Formula 1>. Pharmaceutically acceptable salts of the present invention can include acid addition salt of a compound according to the invention when the compound is fully basic. Such acid addition salt includes salts holding inorganic acid providing pharmaceutically acceptable anion such as hydrogen halide, or organic acid, or salts holding sulfuric acid or phosphoric acid, or salts holding trifluoroacetic acid, citric acid or maleic acid. And, it include for example hydrochlorides, hydrobromides, phosphonates, sulfates, alkylsulfonates, arylsulfonates, acetates, benzoates, citrates, maleates, fumarates, succinates, lactates and tartarates by suitable salts. When a

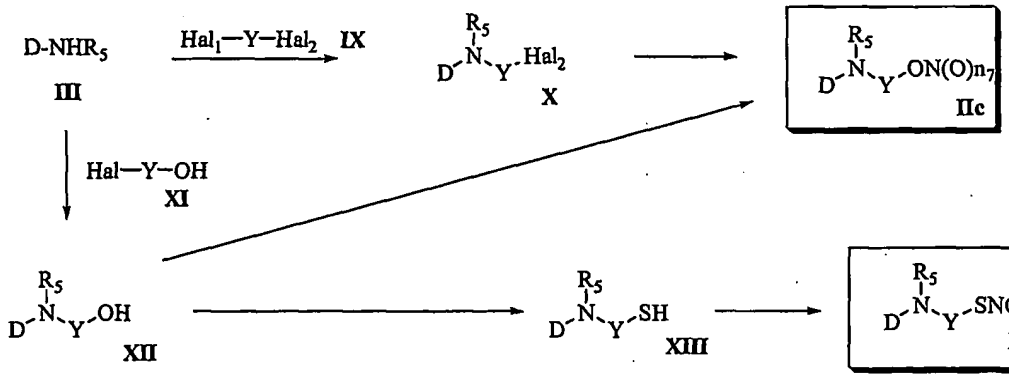
compound of the present invention is fully acidic, pharmaceutically acceptable salts can include inorganic salts or organic salts providing pharmaceutically acceptable cation. Said inorganic salts include sodium salts, potassium salts, calcium salts or magnesium salts, etc., said organic salts include methylamine salts, dimethylamine salts, trimethylamine salts, piperidine salts or morpholine salts, etc.

The present invention also provides a preparation method for tricyclic derivatives represented by the <Formula 1>. The preparation method for tricyclic derivatives of the present invention is described in the below Scheme 1 ~ Scheme 8. Precisely, in the <Formula 1>, when R_1 is $-T_1-B_1$ and B_1 is one of said (a), (b), (c), (d) and (e), the derivatives are prepared according to the method of Scheme 1 ~ Scheme 6. In the meantime, in the <Formula 1>, when R_1 is $-T_1-B_1$ and B_1 is one of said (f), (g), (h), (i) and (j), the derivatives are prepared by the method of Scheme 7 and Scheme 8. And a concrete compound of the <Formula 1> is represented by general formulas (IIa), (IIb), (IIc), (IId), (IIe), (IIf), (IIg), (IIh), (IIi), (IIj), (IIk), (IIl), (IIm), (IIn), (IIo) and (IIp) in Scheme 1 ~ Scheme 8.

<Scheme 1>



<Scheme 2>



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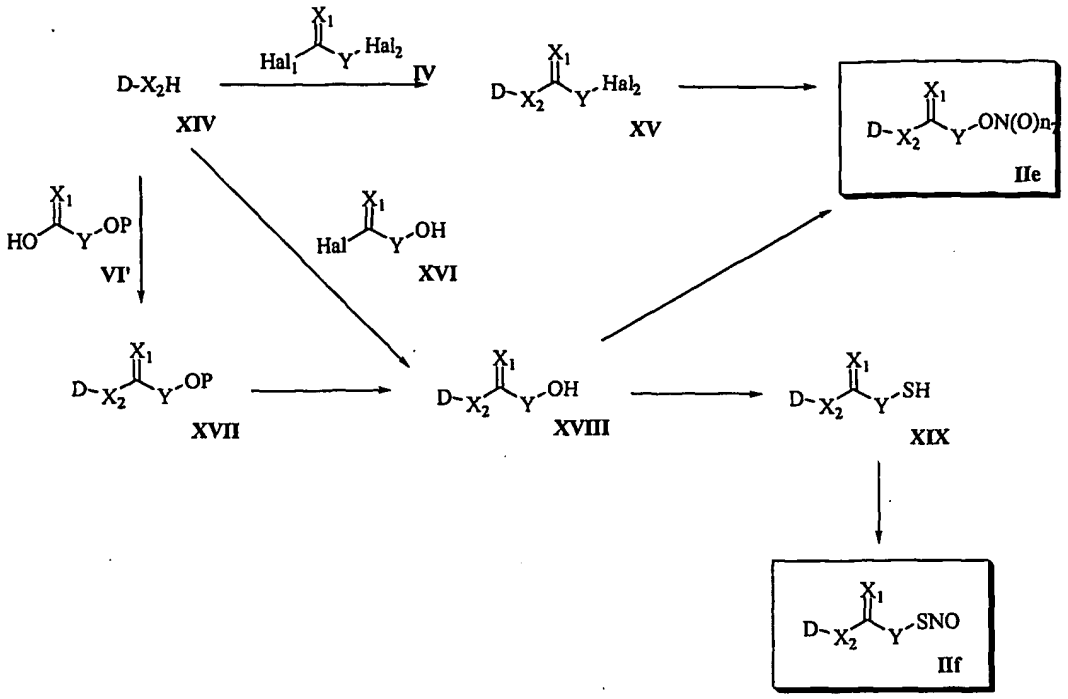

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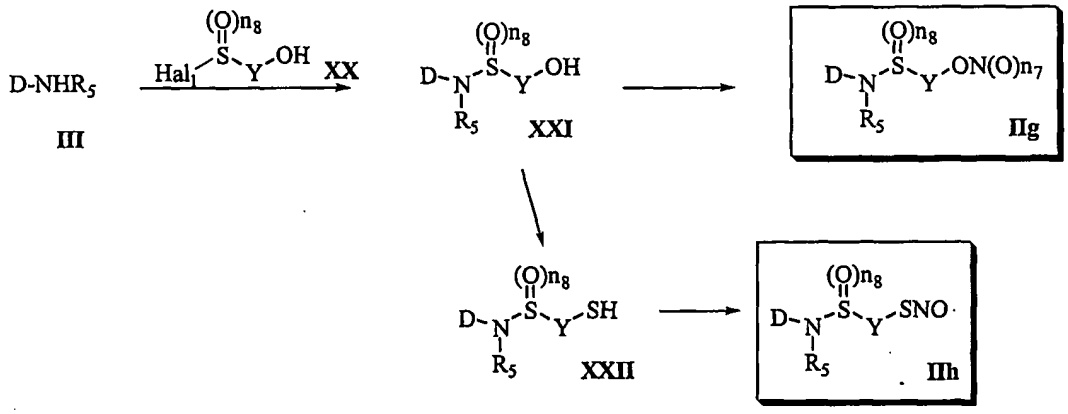
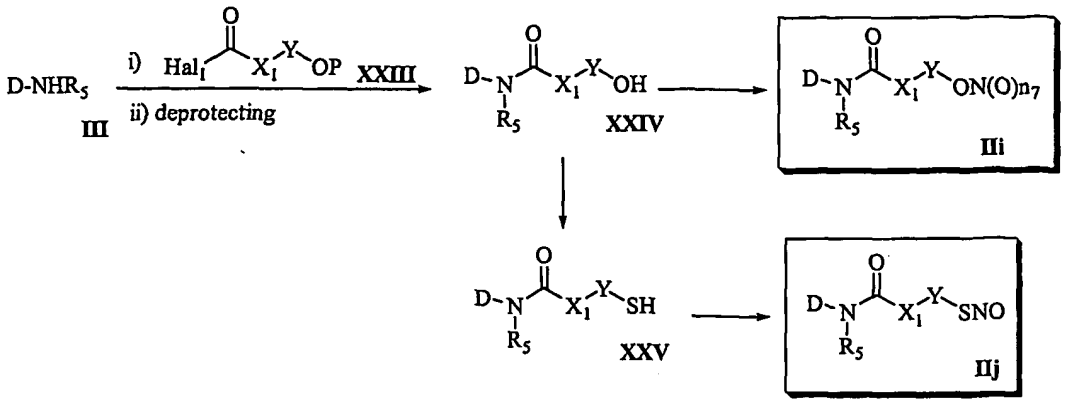


<Scheme 5>



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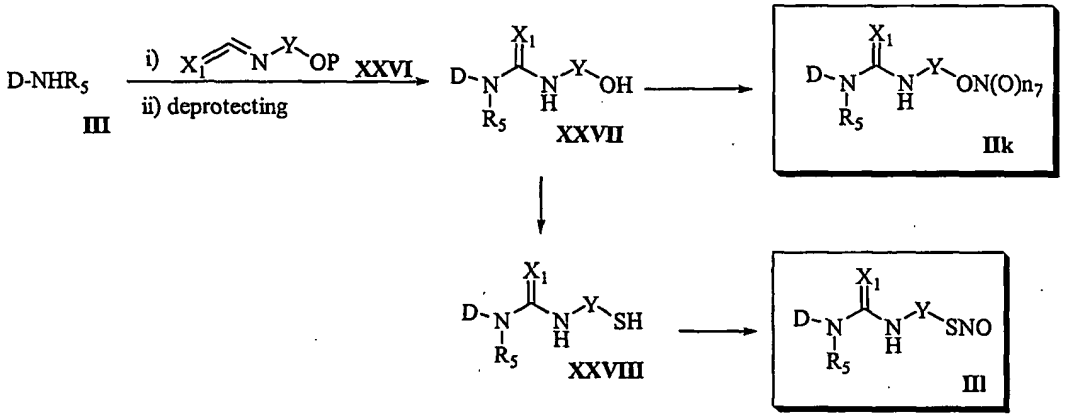

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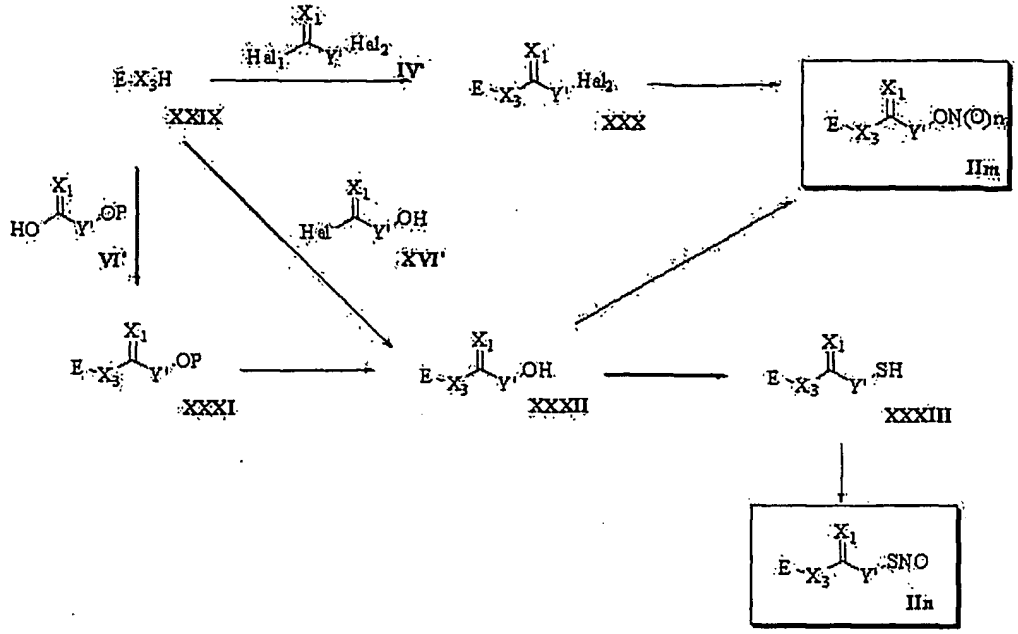
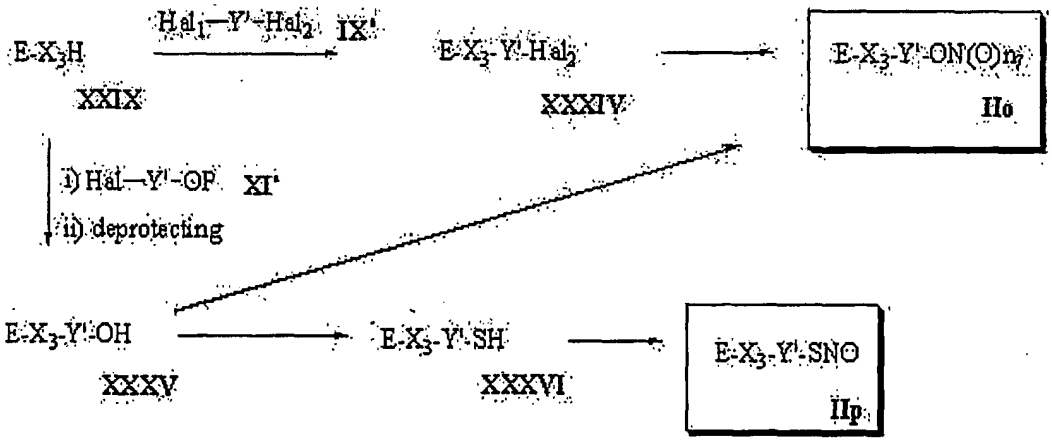
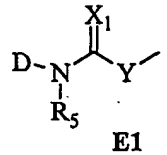
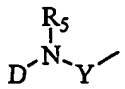


<Scheme 8>



In the above Scheme, E is following E1 ~ E6 respectively;


E1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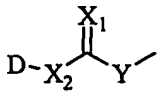
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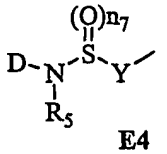
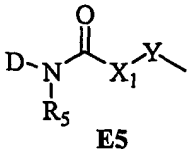
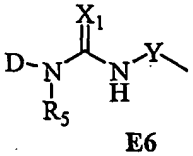
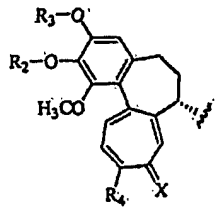

E4


E5

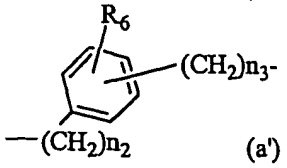

E6

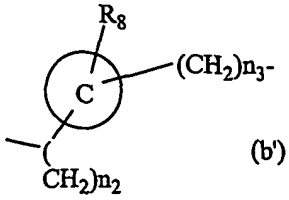
Wherein, X_1, X_2 and X_3 are each O or S.



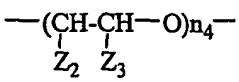
In the above Scheme, D is , and R_2, R_3, R_4 and X are same as defined in the <Formula 1>;

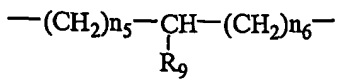
R_5 is H or lower alkyl; X_1, X_2 and X_3 are each independently O or S; Hal_1 and Hal_2 are halogens; Hal_1 and Hal_2 of general formula (IV) and (IX) are each same or different halogens, for example F, Cl, Br or I; P is conventional protecting group of hydroxy such as methoxymethyl, t-butyldimethylsilyl or benzyl; Y and Y' are same or different, and indicate following general formula (a'), (b'), (c'), (d') and (e') respectively,

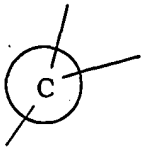

(a')


(b')


(c')


(d')


(e')

Wherein,  , R_6, R_8, R_9, Z_1, Z_2, Z_3, n_2, n_3, n_4, n_5 and n_6 are same as defined in the <Formula 1>, n_7 and n_8 are integers of 1~2.

The preparation method for tricyclic derivatives of the present invention is illustrated more precisely hereinafter.

<Method 1>

According to method 1 of the present invention for the preparation of compounds of formula (11a) and (11b), the compound represented by formula (V) is prepared by amidation reaction making amine compound of formula (III) be reacted with halogen compound of formula (IV), which is step 1. In step 1, a base might be excluded, but the reaction is generally performed with a solvent such as dichloromethane, chloroform,

tetrahydrofuran, diethylether, toluene or dimethylformamide etc., which have no influence on amidation reaction, in the presence of pyridine, triethylamine, diethylisopropylamine or N-methylmorpholine etc., a base that can be acceptable for amidation reaction in general. Reaction temperature is not limited in particular, but generally reaction can performed under cold temperature or elevated temperature, is performed preferably at room temperature.

In step 2, conversion of the compound of formula (V) prepared in the above step 1 to nitrooxy compound (n_7 = 2) of formula (IIa) and to nitrosooxy compound (n_7 = 1) of formula (IIa) was accomplished by nitration reaction and nitrosation reaction, respectively. Nitration reaction needs a compound that is able to convert halogen into nitrate, and is performed using silver nitrate ($AgNO_3$), t-butylammonium nitrate (Bu_4NNO_3), etc., in the presence of chloroform, acetonitrile, a mixture of acetonitrile and aqueous solution, or dichloromethane, which are all solvents not affecting the reaction. Nitrosation reaction might use a compound that is able to convert halogen into nitrosate, too, and is performed preferably using silver nitrite ($AgNO_2$) or sodium nitrite ($NaNO_2$) in the

presence of chloroform, acetonitrile, a mixture of acetonitrile and aqueous solution, aqueous solution, or dichloromethane, which are also solvents not affecting the reaction. Reaction temperature is not limited in particular, but generally reaction can performed under cold temperature or elevated temperature, is performed preferably at room temperature.

Another way to give the compound of formula (IIa) is as follows; reaction of the compound of formula (III) with the compound of formula (VI) is performed to give the compound of formula (VII), and then, conversion of the compound of formula (VII) to the compound of formula (IIa) is accomplished. The reaction of the compound of formula (III) and the compound of formula (VI) is performed in the presence of a coupling agent such as 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide(EDCI), 1-hydroxybenzotriazole hydrate(HOBT) or 1,3-dicyclohexyl carbodiimide(DCC). This reaction might be performed without a base, but generally with a base such as 4-dimethylaminopyridine, pyridine, triethylamine, diethylisopropylamine, N-methylmorpholine or dimethylphenylamine etc., which can be used in amidation reaction, in a solvent having no negative effect on the reaction, for example acetonitrile, dimethylformamide, dichloromethane, etc.

Reaction temperature is not limited in particular, but generally reaction can performed under cold temperature or elevated temperature, is performed preferably at room temperature. Direct conversion of the compound of formula (VII) to the compound of formula (IIa) is accomplished by the reaction of alcohol with triphenylphosphin (PPh_3), N-bromosuccineimide (NBS) and silver nitrate, or silver nitrite. The reaction is performed in a solvent having no effect on the reaction such as chloroform, acetonitrile, dichloromethane, a mixture of acetonitrile and dichloromethane, etc. Reaction temperature is not limited in particular, but generally reaction is performed under cold temperature or at room temperature. Another way for conversion of the compound of formula (VII) to the compound of formula (IIa) is as follows; conversion of the compound of formula (VII) to halogen compound of formula (V) is accomplished first, and then conversion thereof to the compound of formula (IIa) is accomplished again. At this time, the conversion into halogen compound is performed by using a reagent that generally converts hydroxy group to halogen, for example tribromophosphin, tetrabromomethane etc., in the presence of chloroform, acetonitrile, dichloromethane etc., which are solvents having no negative effect on the reaction. Reaction

temperature is not limited in particular, but generally reaction is performed under cold temperature or at room temperature.

Processes for preparing the compound of formula (IIb) of the method 1 of the present invention are as follows; conversion of hydrogen included in alcohol of formula (VII) to a leaving group such as mesylate, tosylate or triplate is accomplished, followed by reaction with potassium thioacetate, to give thioacetate ester compound. Hydrolysis of the compound in the presence of a base is accomplished to give the compound of formula (VIII). At this time, a base is selected among general bases that are able to hydrolyze an ester compound, for example sodium hydroxide, potassium hydroxide or sodium thiomethoxide. And an alcohol solution such as methanol or ethanol is preferred as a solvent for the reaction. Reaction temperature is not limited in particular, but generally reaction can performed under cold temperature or elevated temperature, is performed preferably at room temperature. Reaction of the compound of formula (VIII) with sodium nitrite under an acidic condition, leads to the conversion of the compound to nitrosothio compound of formula (IIb). A solvent for the reaction is selected from a group consisting of methanol, ethanol,

acetonitrile, a mixture of acetonitrile and aqueous solution, or dichloromethane etc., which is not to affect the reaction. Reaction temperature is not limited in particular, but generally reaction can performed under cold temperature or elevated temperature, is performed preferably at room temperature.

<Method 2>

According to method 2 of the present invention, compounds of formula (IIc) and (IId) are prepared. Particularly, in step 1, reaction of the compound of formula (III) with the compound of formula (IX) is accomplished to give the compound of formula (X). This reaction is performed in analogy to the procedure described in method 1 in which conversion of the compound of formula (III) to the compound of formula (V) was accomplished by amidation reaction.

In step 2, conversion of the compound of formula (X) prepared in step 1 to the compound of formula (IIc) is accomplished by nitration reaction along with nitrosation reaction. This reaction is performed in analogy to the procedure described in method 1 in which conversion of the compound of formula (V) to the compound of formula (IIa) was accomplished.

Another way to give the compound of formula (IIc) is as follows; reaction of the compound of formula (III) with the compound of formula (XI) is performed to give the compound of formula (XII), and then, conversion of the compound of formula (XII) to the compound of formula (IIc) is accomplished. Reaction of the compound of formula (XI) with the compound of formula (III) is performed in analogy to the procedure described in method 1 in which conversion of the compound of formula (III) to the compound of formula (V) was accomplished by amidation reaction. Conversion of the compound of formula (XII) to the compound of formula (IIc) is accomplished under the same condition as provided for the conversion of the compound of formula (VII) to the compound of formula (IIa) in method 1.

Conversion of the compound of formula (XII) to the compound of formula (IId) is accomplished under the same condition as provided for the conversion of the compound of formula (VII) to the compound of formula (IIb) in method 1.

<Method 3>

According to method 3 of the present invention, compounds of formula (IIe) and (IIf) are prepared.

Particularly, in step 1, reaction of the compound of formula (XIV) with the compound of formula (IV) is accomplished to give the compound of formula (XV). The reaction in this method is esterification reaction of alcohol (X_2=O) or thioalcohol (X_2=S) with acyl or thioacyl halide, which is performed in the presence of a base that is generally acceptable for esterification reaction. Preferable bases are pyridine, 4-dimethylaminopyridine, triethylamine, diethylisopropylamine, 2,6-lutidine, sodium hydride (NaH), cesium carbonate, or sodium hydroxide and can be used along with a phase transfer catalyst such as benzyltriethylammoniumchloride. Also, above reaction is preferably performed in a solvent having no negative effect on the reaction, for example dichloromethane, chloroform, tetrahydrofuran, diethylether, toluene, dimethylformamide, acetonitrile or aqueous solution. Reaction temperature is not limited in particular, but generally reaction can performed under cold temperature or elevated temperature, is performed preferably at room temperature.

In step 2, conversion of the compound of formula (XV) prepared in step 1 to the compound of formula (IIe) is accomplished by nitration reaction along with nitrosation reaction. This reaction is performed in

analogy to the procedure described in method 1 in which conversion of the compound of formula (V) to the compound of formula (IIa) was accomplished.

Another way to give the compound of formula (IIe) is as follows; reaction of the compound of formula (XIV) with the compound of formula (VI') having a protective group in alcohol group is performed to give the compound of formula (XVII), followed by deprotection reaction to give the compound of formula (XVIII). Conversion of the resultant compound to the compound of formula (IIe) is accomplished. The reaction of the compound of formula (XIV) with the compound of formula (VI') is processed by esterification reaction of alcohol (X_2=O) or thioalcohol (X_2=S) and carboxylic acid or thiocarboxylic acid. The reaction is performed either in an aqueous solution supplemented with an acid such as hydrochloric acid, sulfuric acid, dodecylbenzene sulfonic acid or p-toluenesulfonic acid, at room temperature or under elevated temperature, or under the same condition provided for conversion of the compound of formula (III) to the compound of formula (VII) in method 1. Another esterification reaction is performed by Misunobu reaction using triphenylphosphine and diethyl azodicarboxylate in a solvent not affecting the

reaction. And the solvent is preferably selected from a group consisting of dichloromethane, chloroform, tetrahydrofuran, diethylether, toluene or acetonitrile. Reaction temperature is not limited in particular, but generally reaction is performed under cold temperature or at room temperature. Protecting and deprotecting reaction of alcohol group is performed by known method in general organic synthesis.

Reaction of the compound of formula (XIV) with the compound of formula (XVI) is performed to give the compound of formula (XVIII) in analogy to the procedure described in method 3 in which conversion of the compound of formula (XIV) to the compound of formula (XV) was accomplished.

Conversion of the compound of formula (XVIII) to the compound of formula (IIe) is performed under the same condition provided for conversion of the compound of formula (VII) to the compound of formula (IIa) in method 1.

Conversion of the compound of formula (XVIII) to the compound of formula (IIf) is performed under the same condition provided for conversion of the compound of formula (VII) to the compound of formula (IIb) in method 1.

<Method 4>

According to method 4 of the present invention, compounds of formula (IIg) and (IIh) are prepared. Particularly, in step 1, reaction of the compound of formula (III) with the compound of formula (XX) is accomplished to give the compound of formula (XXI).

When the compound of formula (IIg) is sulfinylamide (n_8=1), the reaction of the compound of formula (III) with sulfinylhalide of formula (XX) is performed without a base or with a base that is applicable to amidation reaction, for example pyridine, triethylamine, diethylisopropylamine, N-methylmorpholine or dimethylphenylamine, in a solvent having no negative effect on the reaction such as dichloromethane, chloroform, tetrahydrofuran, diethylether, toluene or dimethylformamide. Reaction temperature is not limited in particular, but generally reaction can performed under cold temperature or elevated temperature, is performed preferably at room temperature.

When the compound of formula (IIg) is sulfonylamide (n_8=2), the reaction of the compound of formula (III) with sulfonylhalide of formula (XX) is performed either without a base or with a base that is applicable to amidation reaction in general, for

example pyridine, triethylamine, diethylisopropylamine, N-methylmorpholine, sodium hydroxide, sodium carbonate or potassium carbonate, in a solvent having no negative effect on the reaction such as dichloromethane, chloroform, tetrahydrofuran, diethylether, toluene or dimethylformamide. Reaction temperature is not limited in particular, but generally reaction can performed under cold temperature or elevated temperature, is performed preferably at room temperature.

In step 2, conversion of the compound of formula (XXI) prepared in step 1 to the compound of formula (IIg) is performed under the same condition provided for conversion of the compound of formula (VII) to the compound of formula (IIa) in method 1.

Conversion of the compound of formula (XXI) to the compound of formula (IIh) is performed under the same condition provided for conversion of the compound of formula (VII) to the compound of formula (IIb) in method 1.

<Method 5>

According to method 5 of the present invention, compounds of formula (IIi) and formula (IIj) are prepared as follows. In step 1, reaction of the compound of formula (III) with the compound of formula

(XXIII) having a protecting group to alcohol group is performed, followed by deprotection reaction. Conversion of the compound of formula (XXIV) resulted from the above reaction to the compound of formula (IIi) is accomplished. At this time, the reaction of the compound of formula (III) with the compound of formula (XXIII) is performed by using a coupling reagent such as carbonyl dichloride, triphosgen, di-t-butyl dicarbonate or 1,1'-carbonyl diimidazole etc. The reaction can be performed either without a base or with a base that is generally acceptable for amidation reaction, for example pyridine, triethylamine, diethylisopropylamine, N-methylmorpholine or dimethylphenylamine, in a solvent having no negative effect on the reaction such as dichloromethane, chloroform, tetrahydrofuran, diethylether, ethanol or dimethylformamide. Reaction temperature is not limited in particular, but generally reaction is performed under cold temperature or at room temperature. And deprotection reaction is performed by known method in general organic synthesis.

In step 2, conversion of the compound of formula (XXIV) prepared in step 1 to the compound of formula (IIi) is performed under the same condition provided

for conversion of the compound of formula (VII) to the compound of formula (IIa) in method 1.

Conversion of the compound of formula (XXIV) to the compound of formula (IIj) is performed under the same condition provided for conversion of the compound of formula (VII) to the compound of formula (IIb) in method 1.

<Method 6>

According to method 6 of the present invention, compounds of formula (IIk) and formula (III) are prepared as follows. In step 1, reaction of the compound of formula (III) with the compound of formula (XXVI) having a protecting group to alcohol group is performed, followed by deprotection reaction. Then, conversion of the resultant compound of formula (XXVII) to the compound of formula (IIk) is accomplished. At this time, the reaction of the compound of formula (III) with the compound of formula (XXVI) is performed either without a base or with a base that is acceptable for amidation reaction, for example pyridine, triethylamine, diethylisopropylamine or N-methylmorpholine etc, in a solvent having no negative effect on the reaction such as dichloromethane, chloroform, tetrahydrofuran, diethylether, benzene,

acetonitrile, etc. Reaction temperature is not limited in particular, but generally reaction is performed under cold temperature or at room temperature. Protecting and deprotecting reaction of alcohol group is performed by known method in general organic synthesis.

In step 2, conversion of the compound of formula (XXVII) prepared in step 1 to the compound of formula (IIk) is performed under the same condition provided for conversion of the compound of formula (VII) to the compound of formula (IIa) in method 1.

Conversion of the compound of formula (XXVII) to the compound of formula (III) is performed under the same condition provided for conversion of the compound of formula (VII) to the compound of formula (IIb) in method 1.

<Method 7>

According to method 7 of the present invention, compounds of formula (IIm) and formula (IIn) are prepared from the compound of formula (XXIX) in analogy to the procedure described in method 3.

<Method 8>

According to method 8 of the present invention, compounds of formula (IIo) and formula (IIp) are prepared as follows. In step 1, reaction of the compound of formula (XXIX) with the compound of formula (IX') is performed to give a compound of formula (XXXIV). This reaction is performed by etherification reaction of alcohol (X_2=O) or thioalcohol (X_2=S) with alkylhalide in the presence of a base acceptable for etherification reaction. As a base suitable for that purpose, sodium hydride (NaH), t-potassium butoxide (t-BuOK), n-BuLi, sodium hydroxide, potassium hydroxide and a phase transfer catalyst such as benzyltriethylammoniumchloride etc, or crown ether is preferred. The reaction is performed preferably in a solvent having no negative effect on the reaction, for example dichloromethane, chloroform, tetrahydrofuran, diethylether, toluene, dimethylformamide, aqueous solution, dimethylsufoxide or benzene, etc. Reaction temperature is not limited in particular, but generally reaction can performed under cold temperature or elevated temperature, is performed under cold temperature or at room temperature.

In step 2, conversion of the compound of formula (XXXIV), prepared in the above step 1, to the compound of formula (IIo) is accomplished by nitration reaction

or nitrosation reaction. This reaction is performed in analogy to the procedure described in method 1 in which conversion of the compound of formula (V) to the compound of formula (IIa) was accomplished.

Another way to prepare the compound of formula (IIo) is as follows. Reaction of the compound of formula (XXIX) with the compound of formula (XI') having a protecting group to alcohol group is performed, followed by deprotection reaction to give the compound of formula (XXXV). Conversion of the compound of formula (XXXV) to the compound of formula (IIo) is accomplished. Reaction of the compound of formula (XXIX) with the compound of formula (XI') is performed under the same condition given for conversion of the compound of formula (XXIX) to the compound of formula (XXXIV) accomplished by etherification reaction in method 8.

Conversion of the compound of formula (XXXV) to the compound of formula (IIo) is performed under the same condition provided for conversion of the compound of formula (VII) to the compound of formula (IIa) in method 1.

Conversion of the compound of formula (XXXV) to the compound of formula (IIp) is performed under the same condition provided for conversion of the compound

of formula (VII) to the compound of formula (IIb) in method 1.

The target compounds given by the above reactions can be separated and purified by general methods such as column chromatography, recrystallisation, etc.

The present invention provides also a pharmaceutical composition containing tricyclic derivatives represented by the <Formula 1> or pharmaceutically acceptable salts thereof as an effective ingredient.

Tricyclic derivatives according to the present invention or pharmaceutically acceptable salts thereof show very strong cytotoxicity to cancer cell lines but have much less toxicity to test animals than colchicine or taxol injection has.

When tricyclic derivatives of the present invention were administered to a BALB/c nude mouse transplanted with human lung cancer cell line NCI-H460, the size and the weight of a tumor were remarkably decreased in proportion to the dosage.

Tricyclic derivatives of the present invention also have a strong activity of antiangiogenesis in HUVEC cells.

Therefore, tricyclic derivatives of the present invention or pharmaceutically acceptable salts thereof can be effectively used as an anticancer agent, an anti-proliferation agent and an angiogenesis inhibitor.

The composition of the present invention might additionally include, in addition to tricyclic derivatives or pharmaceutically acceptable salts thereof, at least one of active ingredients having the same or similar function to the mentioned tricyclic derivatives or pharmaceutically acceptable salts thereof.

The said tricyclic derivatives or pharmaceutically acceptable salts thereof can be administered orally or parenterally and be prepared in general forms of pharmaceutical formulation. The tricyclic derivatives of the present invention or pharmaceutically acceptable salts thereof can be prepared for oral or parenteral administration by mixing with generally used fillers, extenders, binders, wetting agents, disintegrant, diluents such as surfactants, or excipients. Solid formulations for oral administration are tablets, pills, powders, granules and capsules. These solid formulations are prepared by mixing one or more suitable excipients such

as starch, calcium carbonate, sucrose, lactose and gelatin, etc. Except for the simple excipients, lubricants, for example magnesium stearate, talc, etc, can be used. Liquid formulations for oral administrations are suspensions, solutions, emulsions and syrups, and the above mentioned formulations can contain various excipients such as wetting agents, sweeteners, aromatics and preservatives in addition to generally used simple diluents such as water and liquid paraffin. Formulations for parenteral administration are sterilized aqueous solutions, water-insoluble excipients, suspensions, emulsions, lyophilized agent and suppositories. Water insoluble excipients and suspensions can contain, in addition to the active compound or compounds, propylene glycol, polyethylene glycol, vegetable oil like olive oil, injectable ester like ethylolate, etc. Suppositories can contain witepsol, macrogol, tween 61, cacao butter, laurin butter, glycerol and gelatin.

The composition of the present invention can be prepared for either oral or parenteral administration (for example, intravenous, subcutaneous, intraperitoneal or local injection), and dosage is determined by weight, age, gender, condition of health and diet of a patient and administration method,

excretion rate and severity of a disease. The preferable effective dosage of the tricyclic derivatives of the present invention is 3~300 mg/kg (body weight), and administration times are once or several times per day.

EXAMPLES

Practical and presently preferred embodiments of the present invention are illustrative as shown in the following Examples.

However, the present invention is not limited by following examples.

7-Deacetylcolchicine used in the below examples was prepared by the method described in [EP 0493064; Synthetic Communications 1997, 27(2), 293-296].

7-Amino-1,2,3-trimethoxy-10-methylsulfanyl-6,7-dihydro-5*H*-benzo[a]-heptalen-9-one was prepared by the method described in (WO 9421598; *Bioorganic & Medicinal Chemistry*, Vol 5, No. 12, pp 2277-2282, 1997).

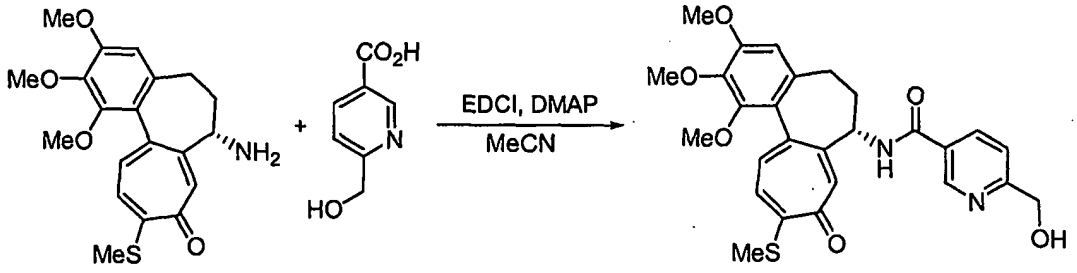
Thiodemecolcine was prepared by the method described in (*J. Med. Chem*, 1985, 28, 1204-1208).

(7S)-7-Amino-3-cyclopentyloxy-1,2-dimethoxy-10-methylsulfanyl-6,7-dihydro-5H-benzo[a]heptalen-9-one,

(7S)-7-amino-3-isopropoxy-1,2-dimethoxy-10-methylsulfanyl-6,7-dihydro-5H-benzo[a]heptalen-9-one, (7S)-7-amino-3-ethoxy-1,2-dimethoxy-10-methylsulfanyl-6,7-dihydro-5H-benzo[a]heptalen-9-one were prepared by the method described in (WO 9611184).

Example 1: Preparation of 6-nitrooxymethyl-*N*-[(7S)-1,2,3-trimethoxy-10-methylsulfanyl-9-oxo-5,6,7,9-tetrahydro-benzo[a]heptalen-7-yl]-nicotineamide

<Step 1> Preparation of 6-hydroxymethyl-*N*-[(7S)-1,2,3-trimethoxy-10-methylsulfanyl-9-oxo-5,6,7,9-tetrahydro-benzo[a]heptalen-7-yl]-nicotineamide

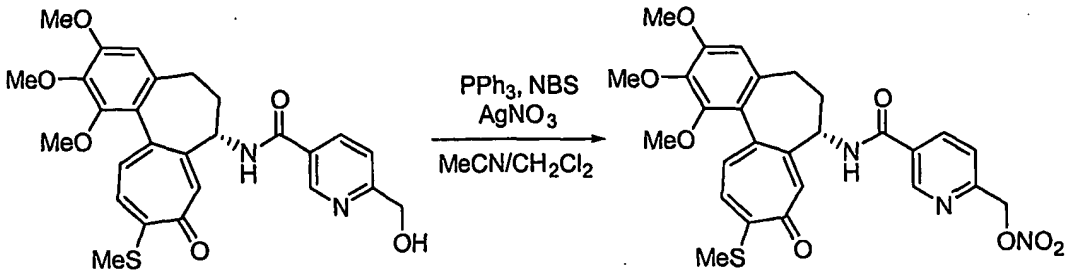


6-Hydroxymethyl-nicotinic acid was synthesized by the method described in (*Bioorg. Med. Chem. Lett*, 1996, *6*, 3025-3028).

To a solution of 7-amino-1,2,3-trimethoxy-10-methylsulfanyl-6,7-dihydro-5*H*-benzo[a]-heptalen-9-one (300 mg, 0.80 mmol), 6-hydroxymethylnicotinic acid (135 mg, 0.88 mmol) and DMAP (60 mg, 0.48 mmol) in 10 ㎖ of acetonitrile was added EDCI (308 mg, 1.60 mmol) at 0℃. The reaction mixture was stirred at room temperature for 2 hours. Water was added to quench the reaction, and aqueous layer was extracted with ethyl acetate. Combined organic layer was dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure. The residue was purified by column chromatography (ethyl acetate:methanol = 8:1), to give 244 mg (yield: 60%, solid having yellow color) of the target compound.

^{1}H NMR (400MHz, $CDCl_3$): δ2.07-2.15(m, 1H), 2.31-2.44(m, 2H), 2.45(s, 3H), 2.56-2.59(m, 1H), 3.75(s, 3H), 3.91(s, 3H), 3.97(s, 3H), 4.66(q, J=10.2Hz, 2H), 4.90-4.93(m, 1H), 6.56(s, 1H), 7.13(t, J=9.1Hz, 2H), 7.40(d, J=10.2Hz, 1H), 7.52(s, 1H), 8.15(dd, J=2.2, 5.8Hz, 1H), 8.80(d, J=6.9Hz, 1H), 8.96(s, 1H)

<Step 2> Preparation of 6-nitrooxymethyl-*N*-[(7S)-1,2,3-trimethoxy-10-methylsulfanyl-9-oxo-5,6,7,9-tetrahydro-benzo[a]heptalen-7-yl]-nicotineamide



The compound (100 mg, 0.19 mmol) prepared in the step 1 of Example 1 and triphenylphosphine (57 mg, 0.21 mmol) were dissolved in acetonitrile/dichloromethane (1.25 ml/0.5 ml), therein NBS (42 mg, 0.23 mmol) was added at -35℃. The reaction mixture was stirred for 20 minutes. Thereafter, therein silver nitrate (40 mg, 0.23 mmol) was slowly added dropwise at room temperature, the reaction mixture was stirred at room temperature for 18 hours. Water was added to quench the reaction, and aqueous layer was extracted with chloroform. Combined organic layer was dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure. The residue was purified by column chromatography (chloroform:methanol = 10:1), to

give 18 mg (yield: 35%, solid having yellow color) of the target compound.

^{1}H NMR (400MHz, $CDCl_3$): δ2.09-2.13(m, 1H), 2.31-2.43(m, 2H), 2.46(s, 3H), 2.55-2.64(m, 1H), 3.75(s, 3H), 3.91(s, 3H), 3.97(s, 3H), 4.93-4.98(m, 1H), 5.50(s, 2H), 6.56(s, 1H), 7.16(t, J=10.9Hz, 1H), 7.26(d, J=8.8Hz, 1H), 7.40(d, J=10.6Hz, 1H), 7.59(s, 1H), 8.27(dd, J=2.2, 5.8Hz, 1H), 8.77(d, J=7.3Hz, 1H), 9.08(s, 1H)

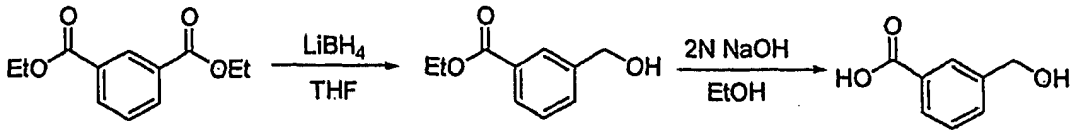
Example 2~4

Compounds of Example 2 - Example 4 were synthesized in analogy to the procedure as described in Example 1, and intermediates were prepared by the method described as follows.

<Intermediate 1> Preparation of 5-hydroxymethyl-furan-2-carboxylic acid

5-Hydroxymethyl-furan-2-carboxylic acid was synthesized by the method described in (*Helv. Chim. Acta*, **1926**, *9*, 1068).

<Intermediate 2> Preparation of 3-hydroxymethylbenzoic acid



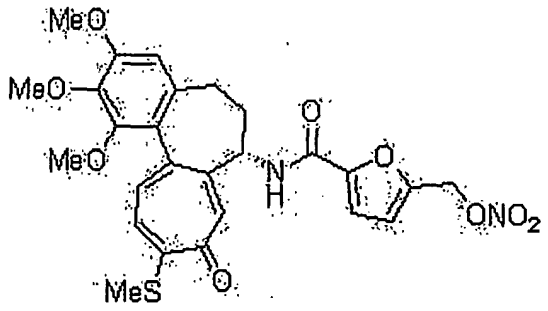
Isophthalic acid diethylester (9.100 g, 40.95 mmol) was dissolved in tetrahydrofuran (20ml). Thereafter, therein lithiumborohydride (11.26 ml, 22.52 mmol, 2M tetrahydrofuran solution) was slowly added dropwise, and the reaction mixture was refluxed for 3 hours. Water was added to quench the reaction, and aqueous layer was extracted with ethyl acetate. Combined organic layer was dried over anhydrous magnesium sulfate, filtered and concentrated under reduced pressure. The residue was purified by column chromatography (hexane:ethyl acetate = 2:1), to give 5.76 g (yield: 77.1%, colorless liquid) of 3-hydroxymethylbenzoic acid ethylester.

Ester compound (1.317 g, 7.311 mmol) prepared above was dissolved in ethanol (6 ml). Thereafter, therein 2*N* NaOH aqueous solution (11.0 ml, 21.93 mmol) was slowly added dropwise, and the reaction mixture was stirred at room temperature for 1 hour. The reaction mixture was neutralized with 1% HCl aqueous solution,

extracted with ethyl acetate, and washed with saturated NaCl solution. Combined organic layer was dried over anhydrous magnesium sulfate, filtered and concentrated under reduced pressure. The residue was purified by column chromatography (ethyl acetate:methyl alcohol = 5:1), to give 1.03 g (yield: 99.2%, white solid) of the target compound.

^{1}H NMR (400MHz, CD_3OD): δ4.66(s, 2H), 7.44(t, *J*=7.7Hz, 1H), 7.58(d, *J*=7.7Hz, 1H), 7.92(d, *J*=7.7Hz, 1H), 8.04(s, 1H)

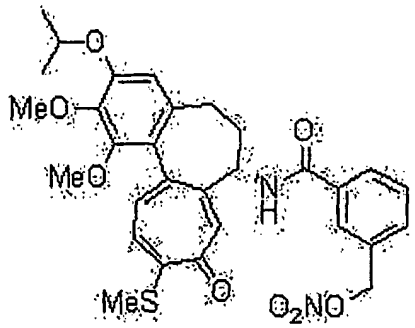
Example 2: 5-nitrooxymethyl-furan-2-carboxylic acid-[(7S)-1,2,3-trimethoxy-10-methylsulfanyl-9-oxo-5,6,7,9-tetrahydro-benzo[a]heptalen-7-yl]-amide



^{1}H NMR (400MHz, $CDCl_3$): δ2.37-2.51(m, 3H), 2.46(s, 3H), 2.61-2.92(m, 1H), 3.74(s, 3H), 3.93(s, 3H), 3.98(s, 3H), 4.82-4.85(m, 1H), 4.85(d, J=13.2Hz, 1H), 4.90(d,

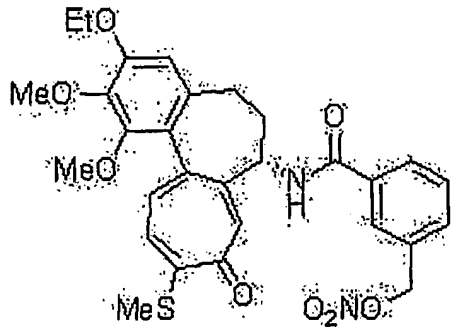
J=13.2Hz, 1H), 6.39(s, 1H), 6.58(s, 1H), 6.64(s, 1H), 7.16(d, J=10.6Hz, 1H), 7.42(d, J=10.2Hz, 1H), 7.72(s, 1H), 8.99(s, 1H)

Example 3: N-[(7S)-3-isopropoxy-1,2-dimethoxy-10-methylsulfanyl-9-oxo-5,6,7,9-tetrahydro-benzo[a]heptalen-7-yl]-3-nitrooxymethyl-benzamide



^{1}H NMR (400MHz, $CDCl_3$): δ1.42(t, J=6.6Hz, 6H), 2.22-2.27(m, 1H), 2.34-2.49(s, 2H), 2.54-2.57(m, 1H), 3.75(s, 3H), 3.96(s, 3H), 4.59-4.63(m, 1H), 4.93-4.97(m, 1H), 5.23(q, J=13.0Hz, 2H), 6.56(s, 1H), 7.15(d, J=10.6Hz, 1H), 7.18-7.25(m, 1H), 7.33(d, J=8.0Hz, 2H), 7.46(d, J=10.2Hz, 1H), 7.64(s, 1H),7.71(d, J=8.0Hz, 1H), 7.88(s, 1H), 8.40(s, 1H)

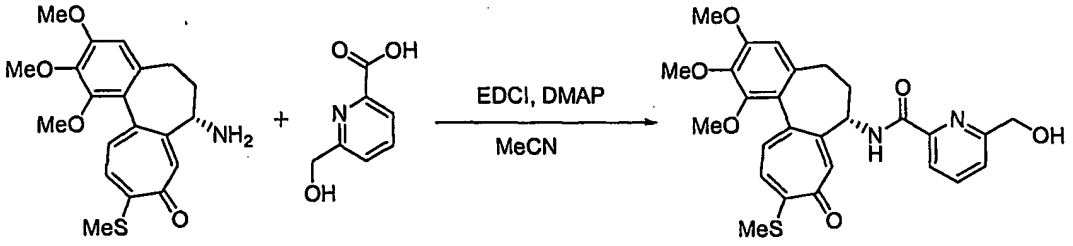
Example 4: N-[(7S)-3-ethoxy-1,2-dimethoxy-10-methylsulfanyl-9-oxo-5,6,7,9-tetrahydro-benzo[a]heptalen-7-yl]-3-nitrooxymethyl-benzamide



^1H NMR (400MHz, $CDCl_3$): δ1.49(t, J=6.9Hz, 3H), 2.17-2.24(m, 1H), 2.34-2.47(m, 2H), 2.45(s, 3H), 2.49-2.58(m, 1H), 3.75(s, 3H), 3.97(s, 3H), 4.12-4.15(m, 2H), 4.89-4.96(m, 1H), 5.24(q, J=12.0Hz, 2H), 6.56(s, 1H), 7.15(d, J=10.2Hz, 1H), 7.21-7.25(m, 1H), 7.31-7.35(m, 1H), 7.41(d, J=10.6Hz, 1H), 7.60(s, 1H), 7.71(d, J=7.3Hz, 1H), 7.78(s, 1H), 8.23(s, 1H)

Example 5: Preparation of 6-nitrooxymethyl-pyridine-2-carboxylic acid-[(7S)-1,2,3-trimethoxy-10-methylsulfanyl-9-oxo-5,6,7,9-tetrahydro-benzo[a]heptalen -7-yl]-amide

<Step 1> Preparation of 6-hydroxymethyl-pyridine-2-carboxylic acid-[(7S)-1,2,3-trimethoxy-10-methylsulfanyl-9-oxo-5,6,7,9-tetrahydro-benzo[a]heptalen -7-yl]-amide

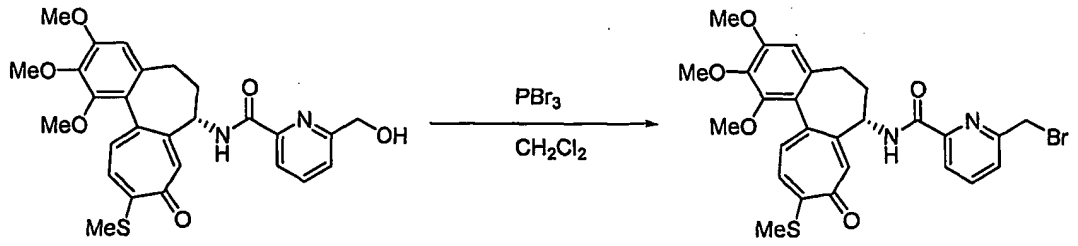


According to the similar procedure in the step 1 of Example 1, by using 6-hydroxymethyl pyridine-2-carboxylic acid (23 mg, 0.17 mmol), 35 mg (yield: 53%, yellow solid) of the target compound was obtained.

^{1}H NMR (400MHz, $CDCl_3$): δ2.45(s, 3H), 2.33-2.50(m, 3H) 2.52-2.73(m, 1H), 3.75(s, 3H), 3.92(s, 3H), 3.97(S,

3H), 4.28(d, J=14Hz, 1H), 4.44(d, J=14Hz, 1H) 4.86-4.92(m, 1H), 6.57(s, 1H), 7.13(d, J=10.4Hz, 1H), 7.37-7.48(m, 4H), 7.73(s, 1H), 9.80(d, J=8Hz, 1H)

<Step 2> Preparation of 6-bromomethyl-pyridine-2-carboxylic acid-[(7S)-1,2,3-trimethoxy-10-methylsulfanyl-9-oxo-5,6,7,9-tetrahydro0benzo[a]heptalen -7-yl]-amide

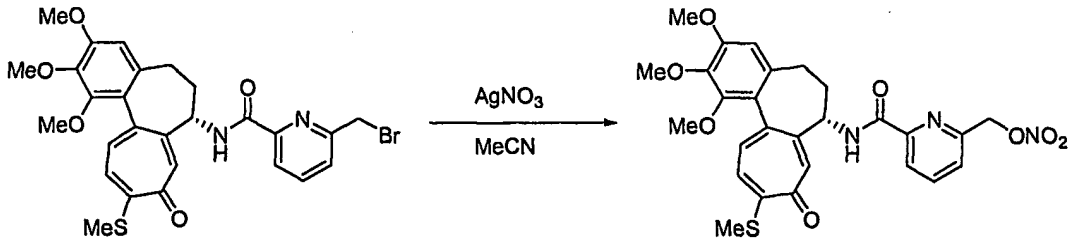


A compound (50 mg, 0.098 mmol) prepared in the step 1 was dissolved in dichloromethane (6 ml). Thereafter, therein tribromophosphine (PBr_3, 0.005 ml, 0.05 mmol) was slowly added dropwise at 0°C, and the reaction mixture was stirred at room temperature for 3 hours. Methanol was added to quench the reaction. Combined organic layer was dried over anhydrous magnesium sulfate, filtered and and concentrated under reduced pressure. The residue was purified by column

chromatography (chloroform:methanol = 99:1), to give 40 mg (yield: 71%, yellow solid) of the target compound.

^{1}H NMR (400MHz, $CDCl_3$): δ2.06-2.14(m, 1H), 2.33-2.41(m, 1H), 2.41(s, 3H), 2.46-2.54(m, 1H) 2.58-2.63(m, 1H), 3.72(s, 3H), 3.90(s, 3H), 3.95(S, 3H), 4.55(d, *J*=2.8Hz, 2H), 4.77-4.83(m, 1H), 6.57(s, 1H), 7.04(d, *J*=10.4Hz, 1H), 7.29(s, 1H), 7.31(d, *J*=10.4Hz, 1H), 7.58(d, *J*=7.6Hz, 1H), 7.78(t, *J*=7.6Hz, 1H), 7.90(d, *J*=8.0Hz, 1H), 8.53(d, *J*=7.6Hz, 1H)

<Step 3> Preparation of 6-nitrooxymethyl-pyridine-2-carboxylic acid-[(7S)-1,2,3-trimethoxy-10-methylsulfanyl-9-oxo-5,6,7,9-tetrahydro-benzo[a]heptalen -7-yl]-amide



A compound (40 mg, 0.070 mmol) prepared in the step 2 was dissolved in acetonitrile (3ml). Thereafter, therein silver nitrate (23 mg, 0.14 mmol) was slowly

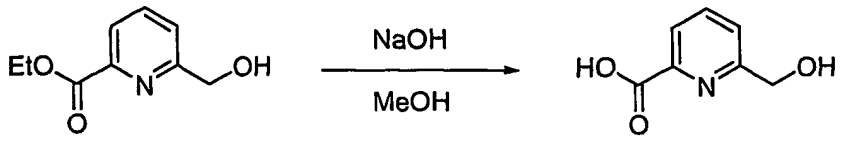
added dropwise, and the reaction mixture was stirred at room temperature for 12 hours. The reaction mixture was washed with water and concentrated under reduced pressure. The residue was purified by column chromatography (chloroform:methanol = 99:1), to give 17 mg (yield: 44.7%, yellow solid) of the target compound.

^{1}H NMR (400MHz, $CDCl_3$): δ2.03-2.10(m, 1H), 2.33-2.41(m, 1H), 2.43(s, 3H), 2.46-2.54(m, 1H) 2.58-2.65(m, 1H), 3.73(s, 3H), 3.92(s, 3H), 3.95(S, 3H), 4.77-4.83(m, 1H), 5.63(d, *J*=3.2Hz, 2H), 6.58(s, 1H), 7.06(d, *J*=10.4Hz, 1H), 7.27(s, 1H), 7.31(d, *J*=10.4Hz, 1H), 7.53(d, *J*=7.6Hz, 1H), 7.88(t, *J*=8.0Hz, 1H), 8.02(d, *J*=7.2Hz, 1H), 8.39(d, *J*=7.2Hz, 1H)

Example 6~24

Compounds of Example 6 - Example 24 were synthesized in analogy to the procedure as described in Example 5, and intermediates were prepared by the method described as follows.

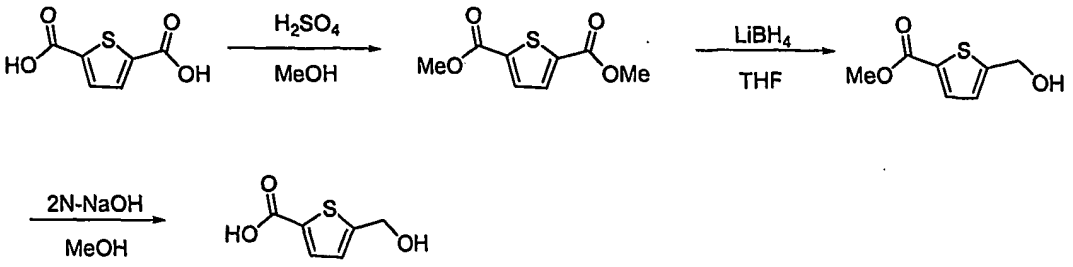
<Intermediate 3> Preparation of 6-hydroxymethyl-pyridine-2-carboxylic acid



6-Hydroxymethyl-pyridine-2-carboxylic acid ethylester (200 mg, 1.1 mmol) (*J. Amer. Chem. Soc*, **1982**, 104, 2251-2257) was dissolved in methanol (1 ml). Thereafter, therein 2*N* NaOH aqueous solution (1 ml) was slowly added dropwise, and the reaction mixture was stirred at room temperature for 1 hour. The reaction mixture was acidified (pH=3) with 2N HCl. Solvent was concentrated under reduced pressure, then, dissolved in methanol and filtered. The filtrate was concentrated under reduced pressure to give 150 mg (yield: 89%, white solid) of the target compound.

^{1}H NMR (400MHz, CD_3OD): δ5.05(s, 2H), 8.34(d, *J*=8.0Hz, 1H), 8.47(d, *J*=8.0Hz, 1H) 8.73(d, *J*=8.0Hz, 1H)

<Intermediate 4> Preparation of 5-hydroxymethyl-thiophene-2-carboxylic acid



Thiophene-2,5-dicarboxylic acid (4 g, 23.3 mmol) was dissolved in methanol (300ml). Catalytic amount of sulfuric acid was slowly added therein. The reaction mixture was refluxed to give 3.8 g (yield: 81.7%, white solid) of thiophene-2,5-dicarboxylic acid dimethylester. The thiophene-2,5-dicarboxylic acid dimethylester (3.7 g, 18.84 mmol) was dissolved in anhydrous tetrahydrofuran (50 ml) at room temperature under a nitrogen atmosphere. 2.0M Lithiumborohydride tetrahydrofuran solution (5.5 ml, 11 mmol) was slowly added therein at 0℃. The reaction mixture was refluxed for 3 hours to give 2.1 g (yield: 64.7%, white solid) of 5-hydroxymethyl-thiophene-2-carboxylic acid methylester. 5-Hydroxymethyl-thiophene-2-carboxylic acid methyl ester (2.1 g, 12.2 mmol) was dissolved in methanol (20 ml). 2N NaOH aqueous solution (15ml) was slowly added therein. The reaction mixture was stirred

at room temperature for 1 hour to give 1.75 g (yield: 89%, white solid) of the target compound.

^{1}H NMR (400MHz, $CDCl_3$): δ3.90(Br, 1H), 4.79(d, J=0.8Hz, 2H), 6.97(d, J=4Hz, 1H), 7.66(d, J=4Hz, 1H)

<Intermediate 5> Preparation of 2-fluoro-3-hydroxymethyl-benzoic acid

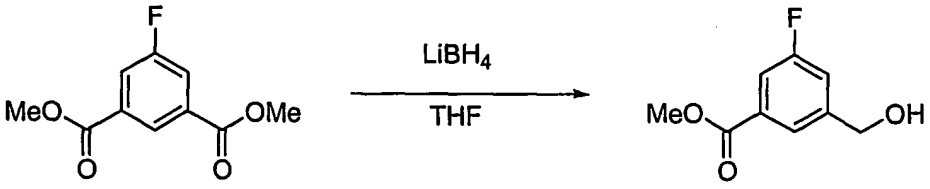
2-Fluoroisophthalic acid (3 g, 16.3 mmol) (*J. Amer. Chem. Soc.*, **1943**, 65, 2308) was dissolved in methanol (150 ml). Catalytic amount of sulfuric acid was slowly added therein. The reaction mixture was refluxed. The resultant was concentrated under reduced pressure to remove solvent, and dissolved in ethyl acetate. Combined organic layer was washed with saturated sodium carbonate, dried over anhydrous sodium sulfate and concentrated under reduced pressure. The residue was purified by column chromatography (ethyl acetate:hexane = 1:2), to give 3.1 g (yield: 88%, white solid) of 2-fluoro-isophthalic acid dimethylester.

Thiophene-2,5-dicarboxylic acid dimethylester (3.1 g, 14.6 mmol) was dissolved in anhydrous tetrahydrofuran (50 ml) at room temperature under a nitrogen atmosphere. 2.0M Lithiumborohydride tetrahydrofuran solution (4.4 ml, 8.7 mmol) was slowly added therein at 0℃. The reaction mixture was refluxed for 3 hours. The reaction mixture was acidified with 1N HCl aqueous solution, concentrated under reduced pressure to remove solvent, and extracted with chloroform. Combined organic layer was dried over anhydrous sodium sulfate, filtered, and solvent was concentrated under reduced pressure. The residue was purified by column chromatography (dichloromethane:methanol = 99:1), to give 1.5 g (yield: 58%, white solid) of 2-fluoro-3-hydroxymethyl-benzoic acid methylester. The 2-fluoro-3-hydroxymethyl-benzoic acid methylester (1.3 g, 7.6 mmol) was dissolved in methanol (20 ml). 2N NaOH aqueous solution (14 ml) was slowly added therein. The reaction mixture was stirred at room temperature for 1 hour. The reaction mixture was acidified (pH=3) with 2N HCl. Solvent was concentrated under reduced pressure, then, dissolved in methanol and filtered. The filtrate was concentrated under reduced pressure to give 1.15 g (yield: 88%, white solid) of the target compound.

^{1}H NMR (400MHz, DMSO-d_6): δ4.58(d, *J*=5.2Hz, 2H),5.37(t, *J*=5.2Hz, 1H) 7.28(t, *J*=8Hz, 1H), 7.68(t, *J*=8Hz, 1H), 7.74(t, *J*=8Hz, 1H)

<Intermediate 6> Preparation of 3-fluoro-5-hydroxymethyl-benzoic acid

<Step 1> Preparation of 3-fluoro-5-hydroxymethyl-benzoic acid methylester

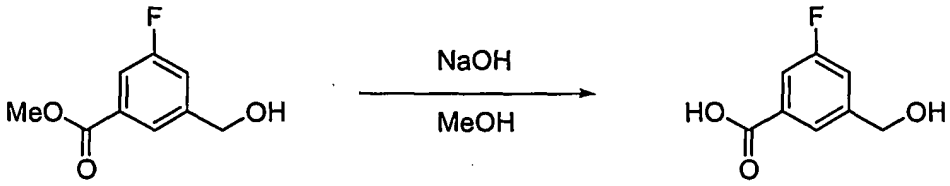


5-Fluoroisophthalic acid dimethylester (1.6 g, 7.54 mmol) (*J. Org. Chem*; 1969, 34, 1960-1961) was dissolved in tetrahydrofuran (15 ml). Thereafter, therein 2.0 M lithiumborohydride tetrahydrofuran solution (2.6 ml, 5.27 mmol) was slowly added dropwise at 0℃, and the reaction mixture was refluxed for 3 hours. The reaction mixture was acidified with 1N HCl aqueous solution, concentrated under reduced pressure to remove solvent, and extracted with chloroform.

Combined organic layer was dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure. The residue was purified by column chromatography (dichloromethane:methanol = 99:1), to give 800 mg (yield: 57%, white solid) of the target compound.

^{1}H NMR (400MHz, $CDCl_3$): δ3.92(d, *J*=1.6Hz, 3H), 4.75(d, *J*=4Hz, 2H), 7.29-7.32(m, 1H), 7.61(dd, *J*=9.2, 1.6Hz, 1H), 7.8(d, *J*=0.8Hz, 1H)

<Step 2> Preparation of 3-fluoro-5-hydroxymethyl-benzoic acid

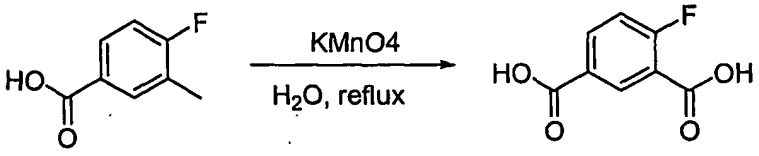


According to the similar procedure in the method of intermediate 3, by using a compound prepared in the step 1, 1.6 g (yield: 94%, white solid) of the target compound was obtained.

^{1}H NMR(400MHz, CD_3OD): δ4.66(d, *J*=0.8Hz, 2H), 7.33-7.36(m, 1H), 7.56-7.59(m, 1H), 7.83-7.84(m, 1H)

<Intermediate 7> Preparation of 4-fluoro-3-hydroxymethyl-benzoic acid

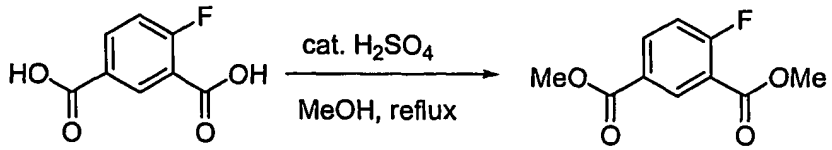
<Step 1> Preparation of 4-fluoro-isophthalic acid



4-Fluoro-3-methyl-benzoic acid (2.52 g, 16.346 mmol) and potassium permanganate (10.33 g, 65.382 mmol) were dissolved in aqueous solution (300 ml), and the mixture was refluxed for 1 day. The reaction mixture was filtered and the resultant solution was cooled down at room temperature, then conc. HCl solution was added thereto. The produced solid was heated until it was completely melted. The temperature was lowered again into room temperature and then the solid was filtered, to give 2.08 g (yield: 69.1%, white solid) of the target compound.

^{1}H NMR (400MHz, CD_3OD): δ7.32(dd, *J*=10.4, 8.6Hz, 1H), 8.21-8.25(m, 1H), 8.59(dd, *J*=7.0, 2.4Hz, 1H)

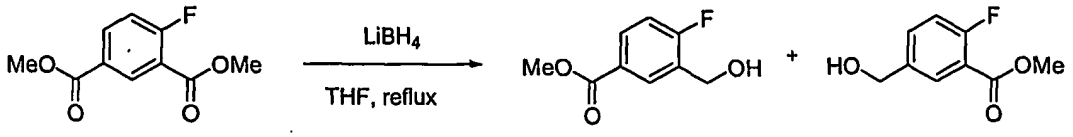
<Step 2> Preparation of 4-fluoro-isophthalic acid dimethylester



A compound prepared in the step 1 (2.08 g, 11.29 mmol) was dissolved in methanol (30 ml). Then, 10 drops of conc. sulfuric acid were added therein. The reaction mixture was refluxed for 1 day, neutralized with saturated sodium hydrogen carbonate aqueous solution, and extracted with chloroform. Combined organic layer was dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure, to give 2.21 g (yield: 92.2%, white solid) of the target compound.

^{1}H NMR (400MHz, $CDCl_3$): δ3.96(s, 3H), 3.97(s, 3H), 7.22(dd, *J*=10.3, 8.8Hz, 1H), 8.20-8.23(m, 1H), 8.64(dd, *J*=7.0, 2.2Hz, 1H)

<Step 3> Preparation of 4-fluoro-3-hydroxymethyl-benzoic acid methylester and 2-fluoro-5-hydroxymethyl-benzoic acid methylester



A compound prepared in the step 2 (104.5 mg, 0.493 mmol) was dissolved in tetrahydrofuran solution (4 ml). Then, 2M lithiumborohydride tetrahydrofuran solution (0.123 ml, 0.246 mmol) was slowly added therein. The reaction mixture was refluxed for 1 day. The reaction was quenched by water. Then, pH was adjusted to 5 with 1M HCl solution at 0℃. Extraction with ethyl acetate was performed. Combined organic layer was dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure. The residue was purified by column chromatography (hexane:ethylacetate = 2:1), to give 4-fluoro-3-hydroxymethyl-benzoic acid methylester (45.4 mg, yield: 50.1%, colorless liquid) and 2-fluoro-5-hydroxymethyl-benzoic acid methylester (15.4 mg, yield: 17.0%, colorless liquid).

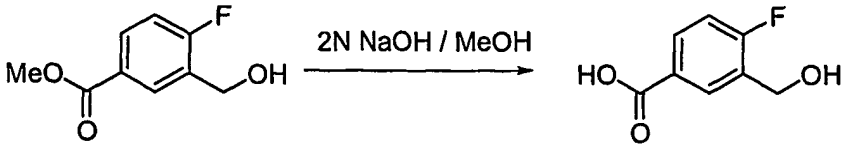
4-fluoro-3-hydroxymethyl-benzoic acid methylester:

^{1}H NMR (400MHz, $CDCl_3$): δ2.73(t, *J*=5.1Hz, 1H), 3.90(s, 3H), 4.78(d, *J*=5.1Hz, 2H), 7.07(dd, *J*=9.2, 9.2Hz, 1H), 7.93-7.97(m, 1H), 8.14(dd, *J*=7.1, 2.2Hz, 1H)

2-fluoro-5-hydroxymethyl-benzoic acid methylester:

^{1}H NMR (400MHz, $CDCl_3$): δ1.96(t, *J*=4.4Hz, 1H), 3.94(s, 3H), 4.70(d, *J*=4.4Hz, 2H), 7.13(dd, *J*=10.6, 8.4Hz, 1H), 7.52-7.56(m, 1H), 7.92(dd, *J*=7.0, 2.2Hz, 1H)

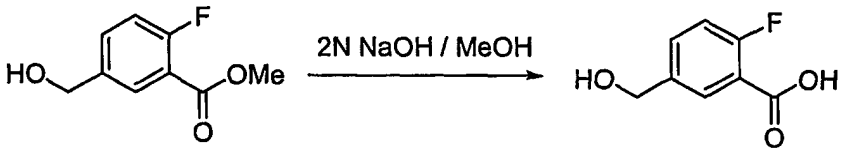
<Step 4> Preparation of 4-fluoro-3-hydroxymethyl-benzoic acid



According to the similar procedure in the method of intermediate 3, by using a compound prepared in the step 3 (1.074 g, 5.380 mmol), 0.906 g (yield: 91.3%, white solid) of the target compound was obtained.

^{1}H NMR (400MHz, CD_3OD): δ4.69(s, 2H), 7.13(dd, *J*=9.9, 8.8Hz, 1H), 7.90-7.97(m, 1H), 8.16(dd, *J*=7.3, 2.2Hz, 1H)

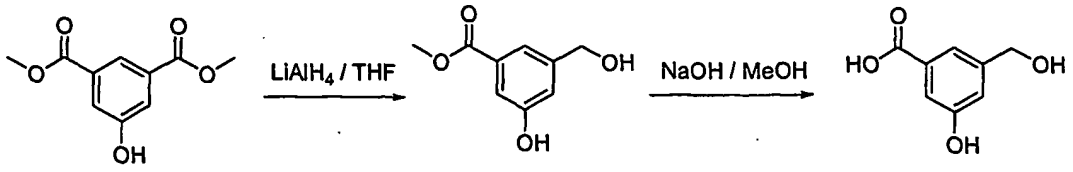
<Intermediate 8> Preparation of 2-fluoro-5-hydroxymethyl-benzoic acid



According to the similar procedure in the method of intermediate 3, by using 2-fluoro-5-hydroxymethyl-benzoic acid methylester prepared in the step 3 of intermediate 7, a target compound was obtained.

^{1}H NMR (400 MHz, CD_3OD): δ4.61(s, 2H), 7.17(dd, *J*=11.0, 8.4Hz, 1H), 7.55-7.59(m, 1H), 7.93(dd, *J*=7.1, 2.4Hz, 1H)

<Intermediate 9> Preparation of 3-hydroxy-5-hydroxymethyl-benzoic acid



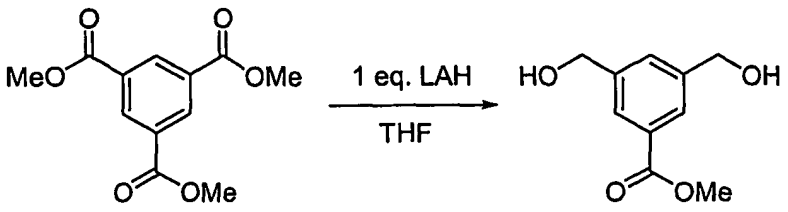
5-Hydroxy-isophthalic acid methylester (300 mg, 1.42 mmol) was dissolved in tetrahydrofuran (20 ml) at 0℃ under a nitrogen atmosphere. Lithium aluminium hydride (30 mg, 0.7 mmol) was added therein. The reaction mixture was stirred at room temperature for 3 hours. Water was added to quench the reaction, and aqueous layer was extracted with ethyl acetate. Combined organic layer was dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure. The residue was purified by column chromatography (dichloromethane:methanol = 20:1). Above-obtained 3-hydroxy-5-hydroxymethyl-benzoic acid methylester (170 mg, 0.93 mmol) was dissolved in methanol (1 ml). 1N NaOH aqueous solution (1 ml) was added therein. The reaction mixture was stirred at room temperature for 1 hour. The reaction mixture was acidified (pH=3) with 2N HCl. Solvent was concentrated under reduced pressure, and then, dissolved in methanol

and filtered. The filtrate was concentrated under reduced pressure to give 150 mg (yield: 96%, white solid) of the target compound.

^{1}H NMR (400MHz, CD_3OD): δ3.87(s, 3H), 4.57(s, 2H), 7.02(s, 1H), 7.31(s, 1H), 7.48(s, 1H)

<Intermediate 10> Preparation of 3,5-*bis*-hydroxymethyl-benzoic acid

<Step 1> Preparation of 3,5-*bis*-hydroxymethyl-benzoic acid methylester

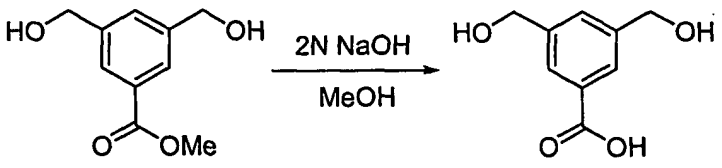


Benzene-1,3,5-tricarboxylic acid trimethylester (1.010 g, 4.003 mmol) was dissolved in tetrahydrofuran (15ml). Thereafter, the temperature was lowered into 0℃, and therein lithium aluminium hydride (0.160 g, 4.003 mmol) was slowly added, and the reaction mixture was stirred at room temperature for 3 hours. Water

(0.15 ml) and 15% NaOH aqueous solution (0.15 ml) were slowly added to quench the reaction. Aqueous solution (0.45 ml) was added again. Solvent was concentrated under reduced pressure. The residue was purified by column chromatography (hexane:ethyl acetate = 1:2), to give 0.34 g (yield: 43.1%, colorless liquid) of the target compound.

^{1}H NMR (400MHz, CD_3OD): δ3.91(s, 3H), 4.66(s, 4H), 7.59(s, 1H), 7.93(s, 2H)

<Step 2> Preparation of 3,5-*bis*-hydroxymethyl-benzoic acid

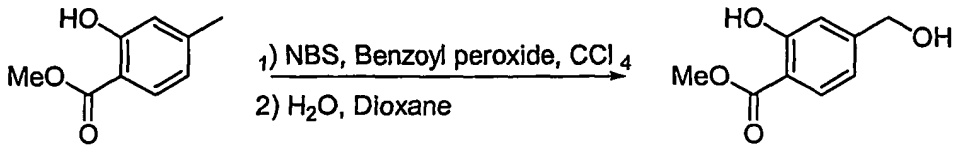


According to the similar procedure in the method of intermediate 3, by using a compound prepared in the step 1 (1.50 g, 7.65 mmol), 0.617 g (yield: 44.3%, white solid) of the target compound was obtained.

^{1}H NMR (400MHz, CD_3OD): δ5.21(s, 4H), 7.55(s, 1H), 7.92(s, 2H)

<Intermediate 11> Preparation of 2-hydroxy-4-hydroxymethyl-benzoic acid

<Step 1> Preparation of 2-hydroxy-4-hydroxymethyl-benzoic acid methylester

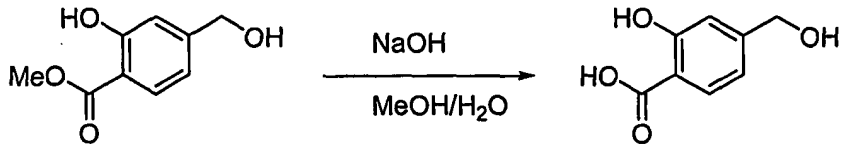


2-Hydroxy-4-methyl-benzoic acid methylester (166 mg, 1 mmol) was dissolved in CCl_4 (1.5ml). NBS (177 mg, 1 mmol) and benzoyl peroxide (5 mg, 0.02 mmol) were added therein. The reaction mixture was stirred at 70℃ for 12 hours. Then, the reaction mixture was washed with water, dried over anhydrous sodium sulfate, and filtered. Solvent was concentrated under reduced pressure. The residue was purified by column chromatography (dichloromethane:hexane = 1:4), to give 4-bromomethyl-2-hydroxy-benzoic acid methylester (130 mg, yield: 53%, white solid). The resultant compound (130 mg, 0.53 mmol) was dissolved in aqueous solution (1.5 ml) and 1,4-dioxane (1.5 ml), and the mixture was stirred at 90℃ for 12 hours. The reaction mixture was

extracted with chloroform. Combined organic layer was dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure. The residue was purified by column chromatography (ethyl acetate:hexane = 1:4), to give 55 mg (yield: 57%, white solid) of the target compound.

^{1}H NMR (400MHz, $CDCl_3$): δ3.95(s, 3H), 4.71(d, *J*=6Hz, 2H), 6.88(d, *J*=8Hz, 1H), 6.99(s, 1H), 7.82(d, *J*=8Hz, 1H), 10.79(s, 1H)

<Step 2> Preparation of 2-hydroxy-4-hydroxymethyl-benzoic acid

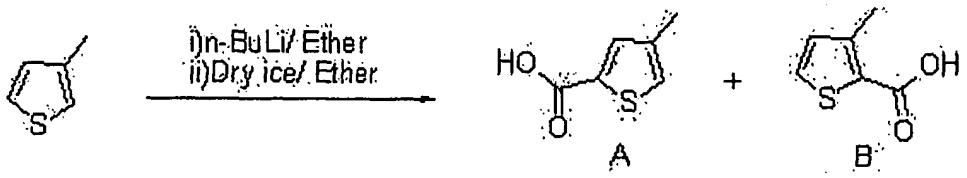


According to the similar procedure in the method of intermediate 3, by using a compound prepared in the step 1, 45 mg (yield: 90%, white solid) of the target compound was obtained.

<Intermediate 12> Preparation of 4-hydroxymethyl-thiophene-2-carboxylic acid

<Step 1> A: 4-methyl-thiophene-2-carboxylic acid,

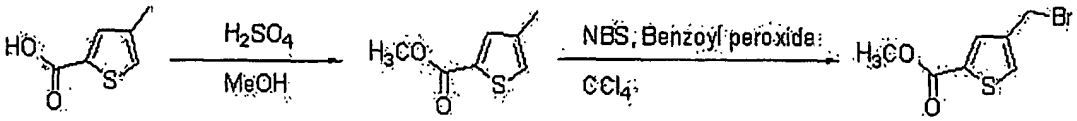
B: Preparation of 3-methyl-thiophene-2-carboxylic acid



2.5M n-Butyllithium (12.2 ml, 30.55 mmol) was dissolved in diethyl ether (1 ml) at room temperature under a nitrogen atmosphere. 3-Methyl-thiophene (3 g, 30.55 mmol) dissolved in diethyl ether was slowly added therein. The mixture was refluxed for 2 hours. Reaction chamber was cooled to 0℃ and dry-ice was slowly added therein. Reaction was quenched with 45 ml of water. Diethyl ether layer was extracted and removed. Water layer was acidified with 1N HCl solution and extracted with ethylacetate. Combined organic layer was dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure. The residue was purified by column chromatography

(hexane:ethyl acetate = 9:1), to give the target compound A (900 mg, white solid) and B (650 mg, white solid).

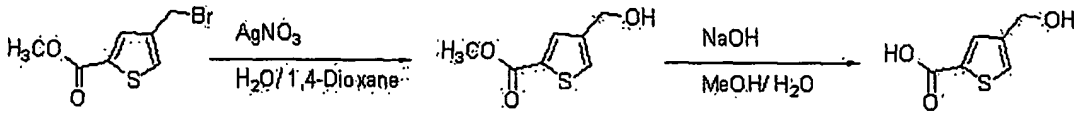
<Step 2> Preparation of 4-bromomethyl-thiophene-2-carboxylic acid methyl ester



4-Methyl-thiophene-2-carboxylic acid (900 mg, 6.33 mmol) prepared in the step 1 was dissolved in methanol (15 ml). Catalytic amount of sulfuric acid was slowly added therein. The reaction mixture was refluxed to give 4-methyl-thiophene-2-carboxylic acid methylester (890 mg, yield: 90%, white solid). 4-methyl-thiophene-2-carboxylic acid methyl ester (200 mg, 1.28 mmol) and NBS (215 mg, 1.216 mmol), and benzoyl peroxide of catalytic amount were dissolved in tetrachloromethane solution (5 ml). The reaction mixture was refluxed (70℃) for 3 hours to give 165 mg (yield: 55%, white solid) of the target compound.

^{1}H NMR(400MHz, $CDCl_3$); δ3.89(s, 3H), 4.46(s, 2H), 7.49(s, 1H), 7.80(s, 1H)

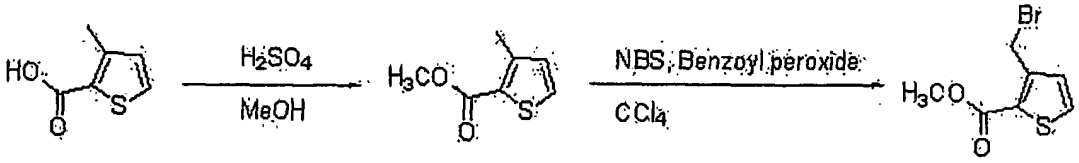
<Step 3> Preparation of 4-hydroxymethyl-thiophene-2-carboxylic acid



A compound (150 mg, 0.638 mmol) prepared in the step 2 was dissolved in 1,4-dioxane (1.5 ml) and water (1.5 ml). Silver nitrate (130 mg, 0.765 mmol) was slowly added therein. The reaction mixture was stirred at room temperature for 12 hours to give 4-hydroxymethyl-thiophene-2-carboxylic acid methyl ester (60 mg, yield: 55%, white solid). This compound (60 mg, 0.348 mmol) was dissolved in methanol (1 ml) at room temperature. 1N NaOH aqueous solution (1 ml) was slowly added therein, and the reaction mixture was stirred at room temperature for 1 hour, to give 50 mg (yield: 95%, white solid) of the target compound.

<Intermediate 13> Preparation of 3-hydroxymethyl-thiophene-2-carboxylic acid

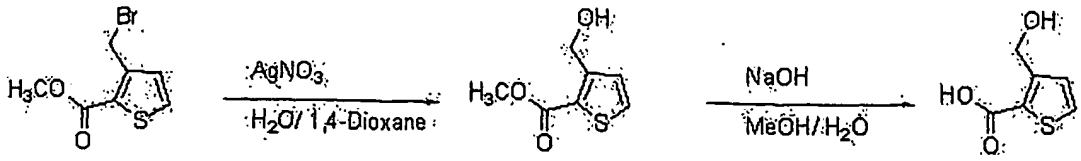
<Step 1> Preparation of 3-bromomethyl-thiophene-2-carboxylic acid methyl ester



According to the similar procedure in the method of step 2 of intermediate 12, by using 3-methyl-thiophene-2-carboxylic acid (650 mg, 4.57 mmol) prepared in the step 1 of intermediate 12, 750 mg (yield: 79%, white solid) of the target compound was obtained.

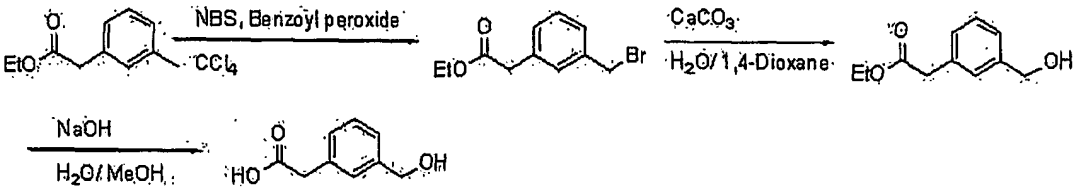
^{1}H NMR(400MHz, $CDCl_3$); δ3.90(s, 3H), 4.91(s, 2H), 7.18(d, J=5.2Hz, 1H), 7.46(d, J=5.2Hz, 1H)

<Step 2> Preparation of 3-hydroxymethyl-thiophene-2-carboxylic acid



According to the similar procedure in the method of step 3 of intermediate 12, by using a compound prepared in the step 1 (750 mg, 3.19 mmol), 210 mg (yield: 92%, white solid) of the target compound was obtained.

<Intermediate 14> Preparation of (3-hydroxymethyl-phenyl)-acetic acid

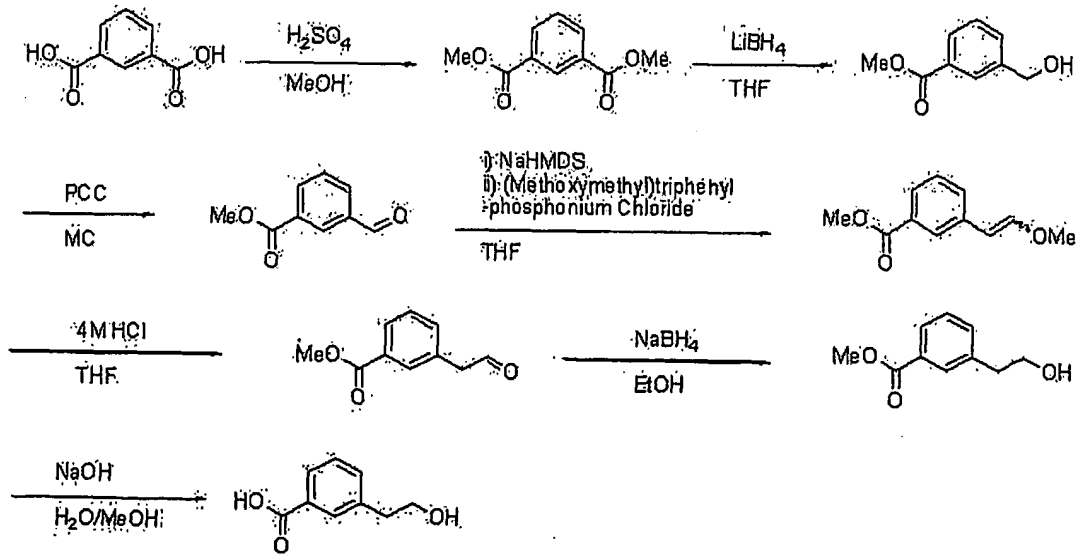


m-Tolyl acetic acid ethyl ester (1 g, 5.6 mmol), NBS (948 mg, 5.33 mmol) and benzoyl peroxide of catalytic amount were dissolved in tetrachloromethane (15 ml). The reaction mixture was refluxed (70℃) for 3 hours to give (3-bromomethyl-phenyl)-acetic acid ethyl ester (600 mg, yield: 42%, white solid). And (3-bromomethyl-phenyl)-acetic acid ethyl ester (130 mg, 0.50 mmol) and calcium carbonate (300 mg, 3 mmol) were

dissolved in water (2 ml) and 1,4-dioxane (2 ml). The reaction mixture was refluxed to give (3-hydroxymethyl-phenyl)-acetic acid ethyl ester (85 mg, yield: 87%, white solid). (3-Hydroxymethyl-phenyl)-acetic acid ethyl ester (85 mg, 0.43 mmol) was dissolved in methanol (1 ml) at room temperature. 1N NaOH aqueous solution (1 ml) was added therein. The reaction mixture was stirred at room temperature for 1 hour, to give 65 mg (yield: 91%, white solid) of the target compound.

^{1}H NMR(400MHz, $CDCl_3$); δ3.66(s, 2H), 4.57(s, 2H), 7.21-7.32(m, 4H)

<Intermediate 15> Preparation of 3-(2-hydroxy-ethyl)-benzoic acid

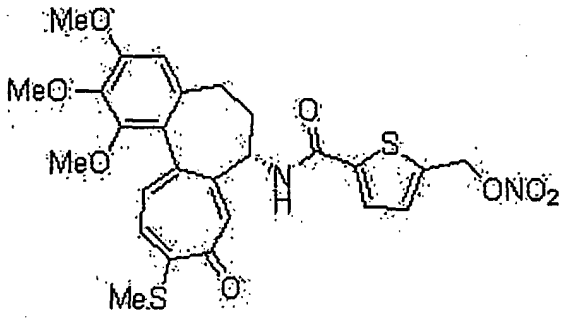


Isophthalic acid (5 g, 30 mmol) was dissolved in methanol (50 ml). Catalytic amount of sulfuric acid was added therein. The reaction mixture was stirred at reflux for 12 hours to give isophthalic acid dimethyl ester (5.2 g, yield: 90%, white solid). Isophthalic acid dimethyl ester (5.2 g, 26.7mmol) was dissolved in tetrahydrofuran (30 ml). 2M Lithiumborohydride tetrahydrofuran (13 ml, 26.7 mmol) was added therein. The reaction mixture was refluxed to give 3-hydroxymethyl-benzoic acid methyl ester (2.7 g, yield: 63%, colorless liquid), and 3-hydroxymethyl-benzoic acid methyl ester (200 mg, 1.20 mmol) was dissolved in dichloromethane (5 ml). PCC (388 mg, 1.8 mmol) was added therein. The reaction mixture was stirred at room temperature for 3 hours to give 3-formyl-benzoic acid methyl ester (140 mg, yield: 72%, white solid). (Methoxymethyl)triphenylphosphonium chloride (770 mg, 2.24 mmol) was dissolved in tetrahydrofuran (5ml) under a nitrogen atmosphere. 1M NaHMDS tetrahydrofuran (2 ml, 2.04 mmol) was slowly added therein at -78℃, and the reaction mixture was stirred for 1 hour. 3-Formyl-benzoic acid methyl ester (160 mg, 0.975 mmol) dissolved in tetrahydrofuran (2 ml) was slowly added therein. The reaction mixture was stirred at room

temperature for 24 hours to give 3-(2-methoxyvinyl)-benzoic acid methyl ester (121 mg, yield: 65%, white solid). 3-(2-Methoxyvinyl)-benzoic acid methyl ester (120 mg, 0.63 mmol) was dissolved in tetrahydrofuran (3 ml). 4M HCl (2 ml) was added therein, and the mixture was stirred at room temperature for 24 hours, to give 3-(2-oxo-ethyl)-benzoic acid methyl ester (60 mg, yield: 54%, white solid). 3-(2-Oxo-ethyl)-benzoic acid methyl ester (60 mg, 0.036 mmol) was dissolved in ethanol (2 ml). $NaBH_4$ (25 mg, 0.67 mmol) was added therein at 0℃, and the mixture was stirred at 1 hour, to give 3-(2-hydroxyethyl)-benzoic acid methyl ester (50 mg, yield: 84%, white solid). 3-(2-Hydroxyethyl)-benzoic acid methyl ester (50 mg, 0.28 mmol) was dissolved in methanol (1 ml). 1N NaOH aqueous solution (1 ml) was added therein. The reaction mixture was stirred at room temperature for 1 hour to give 43 mg (yield: 95%, white solid) of the target compound.

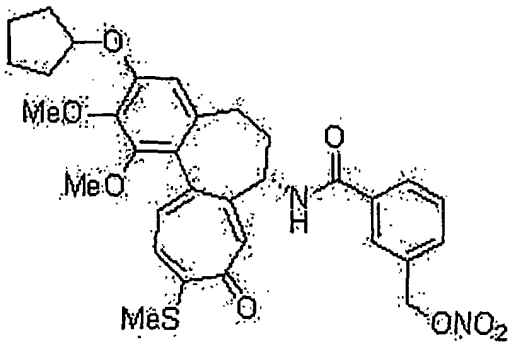
^1H NMR(400MHz, $CDCl_3$); δ2.32(br, 1H), 2.89(t, J=6.4Hz, 2H), 3.85(t, J=6.4Hz, 2H), 7.36(t, J=7.6Hz, 1H), 7.42(d, J=5.6Hz, 1H), 7.88(d, J=8.8Hz, 1H), 7.89(s, 1H)

Example 6: 5-nitrooxymethyl-thiophene-2-carboxylic acid-[(7S)-1,2,3-trimethoxy-10-methylsulfanyl-9-oxo-5,6,7,9-tetrahydro-benzo[a]heptalen-7-yl]-amide



^{1}H NMR(400MHz, $CDCl_3$); δ2.07-2.11(m, 1H), 2.32-2.45(m, 2H), 2.45(s, 3H), 2.53-2.56(m, 1H), 3.71(s, 3H), 3.91(s, 3H), 3.96(S, 3H), 4.84-4.91(m, 1H), 5.46(s, 2H), 6.56(s, 1H), 6.95(d, *J*=3.6Hz, 1H), 7.13(d, *J*=10.8Hz, 1H), 7.37(d, *J*=10.8Hz, 1H), 7.58(d, *J*=4.0Hz, 1H), 7.61(s, 1H), 8.50(d, *J*=6.8Hz, 1H)

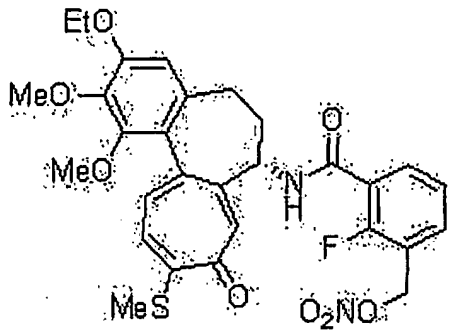
Example 7: N-[(7S)-3-cyclopentyloxy-1,2-dimethoxy-10-methylsulfanyl-9-oxo-5,6,7,9-tetrahydro-benzo[a]heptalen-7-yl]-3-nitrooxymethyl-benzamide



^{1}H NMR(400MHz, $CDCl_3$); δ1.61-1.73(m, 2H), 1.85-2.00(m, 6H), 2.21-2.65(m, 4H), 2.44(s, 3H), 3.75(s, 3H), 3.93(s, 3H), 4.82-4.88(m, 1H), 4.92-5.00(m, 1H), 5.16(dd, *J*=12, 31.2Hz, 2H), 6.55(s, 1H), 7.13-7.17(m, 2H), 7.28(d, *J*=6.4Hz, 1H), 7.41(d, *J*=10.8Hz, 1H), 7.68-7.70(m, 1H), 7.72(s, 1H), 7.78(s, 1H), 8.78(d, *J*=7.2Hz, 1H)

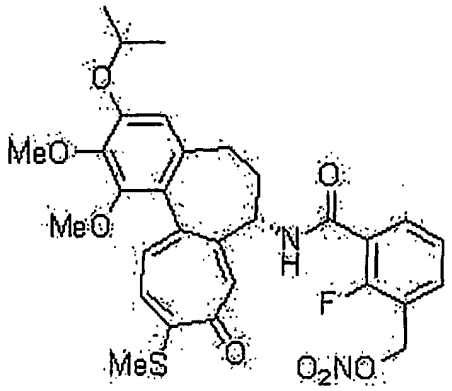
Example 8: N-[(7S)-3-ethoxy-1,2-dimethoxy-10-methylsulfanyl-9-oxo-5,6,7,9-tetrahydro-

benzo[a]heptalen-7-yl]-2-fluoro-3-nitrooxymethyl-benzamide



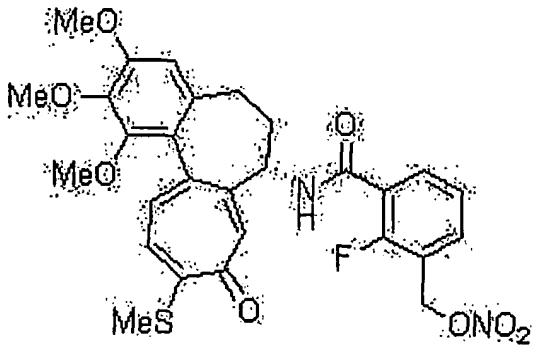
^{1}H NMR(400MHz, $CDCl_3$); δ1.49(t, *J*=6.9Hz, 3H), 1.93-1.98(m, 1H), 2.31-2.39(m, 1H), 2.43(s, 3H), 2.46-2.49(m, 1H), 2.51-2.59(m, 1H), 3.73(s, 3H), 3.96(s, 3H), 4.11-4.14(m, 2H), 4.83-4.86(m, 1H), 5.53(d, *J*=12.8Hz, 1H), 5.59(d, *J*=12.4Hz, 1H), 6.56(s, 1H), 7.07(d, *J*=10.2Hz, 1H), 7.10-7.15(m, 1H), 7.20-7.28(m, 1H), 7.31(d, *J*=10.2Hz, 1H), 7.57(t, *J*=6.4Hz, 1H), 7.97(t, *J*=6.9Hz, 1H)

Example 9: 2-fluoro-*N*-[(7S)-3-isopropoxy-1,2-dimethoxy-10-methylsulfanyl-9-oxo-5,6,7,9-tetrahydro-benzo[a]heptalen-7-yl]-3-nitrooxymethyl-benzamide



^{1}H NMR(400MHz, $CDCl_3$); δ1.41(q, *J*=6.0Hz, 6H), 1.92-1.99(m, 1H), 2.31-2.40(m, 1H), 2.43(s, 3H), 2.46-2.52(m, 1H), 2.55-2.60(m, 1H), 3.72(s, 3H), 3.94(s, 3H), 4.57-4.63(m, 1H), 4.83-4.89(m, 1H), 5.53(d, *J*=12.8Hz, 1H), 5.59(d, *J*=12.4Hz, 1H), 6.57(s, 1H), 7.07(d, *J*=10.6Hz, 1H), 7.15-7.19(m, 1H), 7.23-7.27(m, 1H), 7.34(d, *J*=10.2Hz, 1H), 7.56(t, *J*=7.1Hz, 1H), 7.7(t, *J*=7.5Hz, 1H)

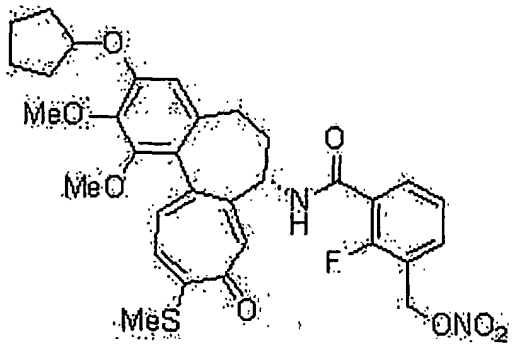
Example 10: 2-fluoro-3-nitrooxymethyl-*N*-[(7S)-1,2,3-trimethoxy-10-methylsulfanyl-9-oxo-5,6,7,9-tetrahydro-benzo[a]heptalen-7-yl]-benzamide



^{1}H NMR(400MHz, $CDCl_3$); δ1.95-2.02(m, 1H), 2.31-2.54(m, 2H), 2.43(s, 3H), 2.59-2.63(m, 1H), 3.73(s, 3H), 3.87(s, 3H), 3.92(s, 3H), 4.82-4.88(m, 1H), 5.51(d, *J*=12.8Hz, 1H), 5.60(d, *J*=12.8Hz, 1H), 6.58(s, 1H), 7.06(d, *J*=10.6Hz, 1H), 7.22-7.27(m, 2H), 7.28(s, 1H), 7.32(d, *J*=10.6Hz, 1H), 7.54-7.58(m, 1H), 7.93-7.98(m, 1H)

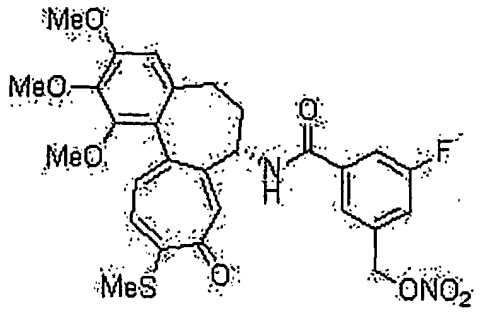
Example 11: N-[(7S)-3-cyclopentyloxy-1,2-dimethoxy-10-methylsulfanyl-9-oxo-5,6,7,9-tetrahydro-

benzo[a]heptalen-7-yl]-2-fluoro-3-nitrooxymethyl-benzamide



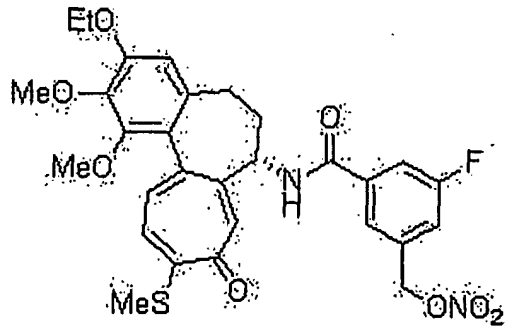
^{1}H NMR(400MHz, $CDCl_3$); δ1.61-1.73(m, 2H), 1.85-2.00(m, 6H), 2.35-2.52(m, 4H), 2.42(s, 3H), 3.72(s, 3H), 3.93(s, 3H), 4.82-4.88(m, 2H), 5.57(dd, *J*=12.8, 37.6Hz, 2H), 6.55(s, 1H), 7.05(d, *J*=10.4Hz, 1H), 7.09-7.13(m, 1H), 7.26(d, *J*=7.2Hz, 2H), 7.32(d, *J*=10.4Hz, 1H), 7.55-7.59(m, 1H), 7.96-8.00(m, 1H)

Example 12: 3-fluoro-5-nitrooxymethyl-*N*-[(7S)-1,2,3-trimethoxy-10-methylsulfanyl-9-oxo-5,6,7,9-tetrahydro-benzo[a]heptalen-7-yl]-benzamide



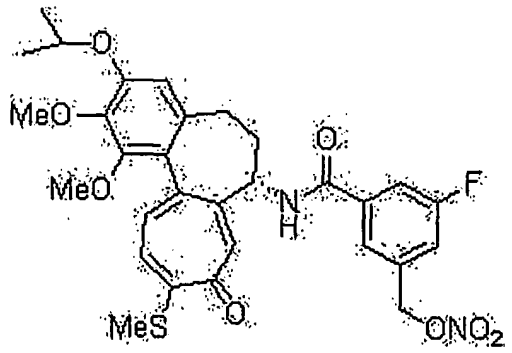
^{1}H NMR(400MHz, $CDCl_3$); δ2.31-2.45(m, 3H), 2.46(s, 3H), 2.59-2.63(m, 1H), 3.75(s, 3H), 3.93(s, 3H), 3.98(S, 3H), 4.90-4.95(m, 1H), 5.11(dd, *J*=12.8, 45.6Hz, 2H), 6.57(s, 1H), 7.03(d, *J*=7.6Hz,1H), 7.19(d, *J*=8.8Hz, 1H), 7.30-7.33(m, 1H), 7.42(d, *J*=10.8Hz, 1H), 7.55(s, 1H), 7.68(s, 1H), 8.76(d, *J*=7.2Hz, 1H)

Example 13: N-[(7S)-3-ethoxy-1,2-dimethoxy-10-methylsulfanyl-9-oxo-5,6,7,9-tetrahydro-benzo[a]heptalen-7-yl)-3-fluoro-5-nitrooxymethyl-benzamide



^{1}H NMR(400MHz, $CDCl_3$); δ1.50(t, J=6.9, 3H), 2.33~2.42(m, 3H), 2.46(s, 3H), 2.51~2.59(m, 1H), 3.75(s, 3H), 3.98(s, 3H), 4.11~4.16(m, 2H), 4.90~4.93(m, 1H), 5.05(d, J=12.4, 1H), 5.17(d, J=12.4, 1H), 6.56(s, 1H), 7.03(d, J=7.3, 1H), 7.20(d, J=10.6, 1H), 7.31(d, J=9.1, 1H), 7.45(d, J=10.6, 1H), 7.55(s, 1H), 7.69(s, 1H), 8.88(d, J=7.3, 1H)

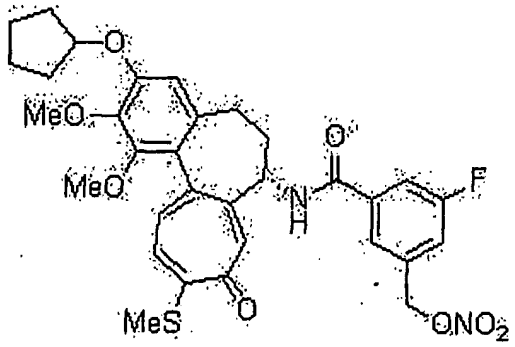
Example 14: 3-fluoro-N-[(7S)-3-isopropoxy-1,2-dimethoxy-10-methylsulfanyl-9-oxo-5,6,7,9-tetrahydro-benzo[a]heptalen-7-yl]-5-nitrooxymethyl-benzamide



^{1}H NMR(400MHz, $CDCl_3$); δ1.37~1.44(m, 6H), 2.34~2.41(m, 3H), 2.46(s, 3H), 2.51~2.58(m, 1H), 3.75(s, 3H), 3.96(s, 3H), 4.57~4.62(m, 1H), 4.91~4.95(m, 1H), 5.05(d, J=12.4, 1H), 5.17(d, J=12.4, 1H), 6.56(s, 1H), 7.03(d, J=8.4, 1H), 7.20(d, J=10.6, 1H), 7.31(d, J=7.3,

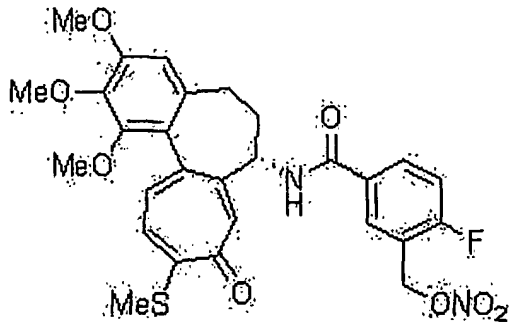
1H), 7.45(d, J=10.6, 1H), 7.55(s, 1H), 7.70(s, 1H), 8.91(d, J=7.3, 1H)

Example 15: *N*-[(7S)-3-cyclopentyloxy-1,2-dimethoxy-10-methylsulfanyl-9-oxo-5,6,7,9-tetrahydro-benzo[a]heptalen-7-yl]-3-fluoro-5-nitrooxymethyl-benzamide



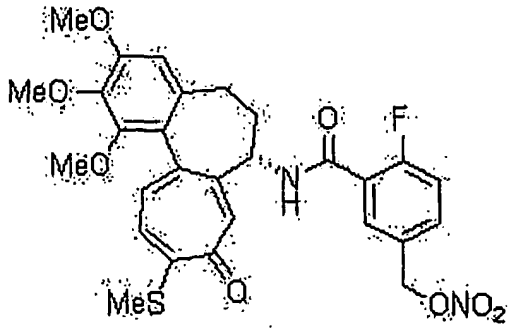
^{1}H NMR(400MHz, $CDCl_3$); δ1.64~1.69(m, 2H), 1.83~1.98(m, 6H), 2.35~2.43(m, 3H), 2.46(s, 3H), 2.51~2.58(m, 1H), 3.74(s, 3H), 3.94(s, 3H), 4.82~4.84(m, 1H), 4.91~4.94(m, 1H), 5.04(d, J=12.4, 1H), 5.16(d, J=12.4, 1H), 6.55(s, 1H), 7.02(d, J=8.4, 1H), 7.20(d, J=10.6, 1H), 7.31(d, J=7.3, 1H), 7.45(d, J=10.2, 1H), 7.55(s, 1H), 7.70(s, 1H), 8.93(d, J=6.9, 1H)

Example 16: 4-fluoro-3-nitrooxymethyl-*N*-[(7S)-1,2,3-trimethoxy-10-methylsulfanyl-9-oxo-5,6,7,9-tetrahydro-benzo[a]heptalen-7-yl]-benzamide



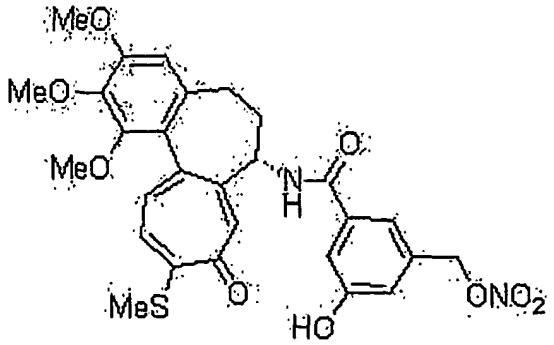
^{1}H NMR(400MHz, $CDCl_3$); δ2.22-2.51(m, 3H), 2.46(s, 3H), 2.55-2.63(m, 1H), 3.76(s, 3H), 3.92(s, 3H), 3.98(s, 3H), 4.90-4.97(m, 1H), 5.10(d, *J*=12.1Hz, 1H), 5.35(d, *J*=12.1Hz, 1H), 6.57(s, 1H), 6.86(dd, *J*=9.2, 8.8Hz, 1H), 7.18(d, *J*=10.3Hz, 1H), 7.43(d, *J*=10.3Hz, 1H), 7.72(s, 1H), 7.76-7.80(m, 1H), 7.91(dd, *J*=6.80, 2.2Hz, 1H), 8.93(d, *J*=7.2Hz, 1H)

Example 17: 2-fluoro-5-nitrooxymethyl-*N*-[(7S)-1,2,3-trimethoxy-10-methylsulfanyl-9-oxo-5,6,7,9-tetrahydro-benzo[a]heptalen-7-yl]-benzamide



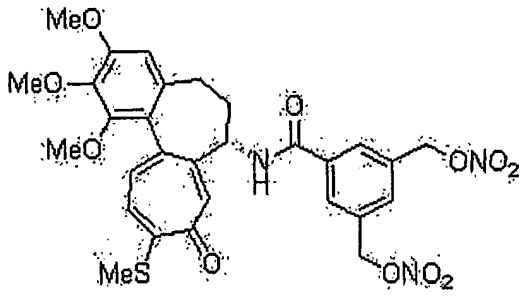
^{1}H NMR(400MHz, $CDCl_3$); δ1.94-2.01(m, 1H), 2.31-2.54(m, 2H), 2.43(s, 3H), 2.58-2.63(m, 1H), 3.73(s, 3H), 3.92(s, 3H), 3.97(s, 3H), 4.81-4.87(m, 1H), 5.36(s, 2H), 6.57(s, 1H), 7.06(d, *J*=10.3Hz, 1H), 7.16-7.28(m, 2H), 7.26(s, 1H), 7.33(d, *J*=10.3Hz, 1H), 7.51-7.55(m, 1H), 7.99(dd, *J*=7.3, 2.6Hz, 1H)

Example 18: 3-hydroxy-5-nitrooxymethyl-*N*-[(7S)-1,2,3-trimethoxy-10-methylsulfanyl-9-oxo-5,6,7,9-tetrahydro-benzo[a]heptalen-7-yl]-benzamide



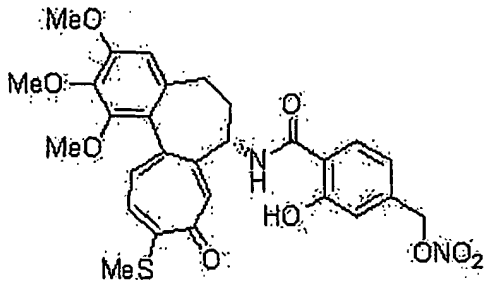
^{1}H NMR(400MHz, $CDCl_3$); δ2.30-2.43(m, 6H), 2.56-2.57(m, 1H), 3.61(s, 3H), 3.90(s, 3H), 3.92(s, 3H), 4.82-4.85(m, 1H), 5.00(dd, *J*=30.8Hz, 12.8Hz, 2H), 6.56(s, 1H), 6.62(s, 1H), 7.11(s, 2H), 7.16-7.19(m, 2H), 7.41(d, *J*=10.8Hz, 1H), 7.66(s, 1H), 8.57(brs, 1H, NH), 8.78(brs, 1H, OH)

Example 19: 3,5-*bis*-nitrooxymethyl-*N*-[(7S)-1,2,3-trimethoxy-10-methylsulfanyl-9-oxo-5,6,7,9-tetrahydro-benzo[a]heptalen-7-yl]-benzamide



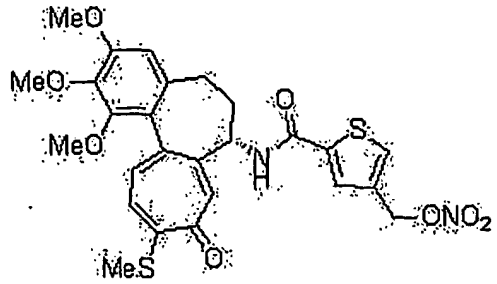
^{1}H NMR(400MHz, $CDCl_3$); δ2.29-2.51(m, 3H), 2.46(s, 3H), 2.60-2.62(m, 1H), 3.77(s, 3H), 3.93(s, 3H), 3.99(s, 3H), 4.94-4.98(m, 1H), 5.18(s, 4H), 6.58(s, 1H), 7.20(d, *J*=10.6Hz, 1H), 7.26(s, 1H), 7.45(d, *J*=10.6Hz, 1H), 7.77(s, 2H), 7.81(s, 1H), 9.12(d, *J*=7.0Hz, 1H)

Example 20: 2-hydroxy-4-nitrooxymethyl-N-[(7S)-1,2,3-trimethoxy-10-methylsulfanyl-9-oxo-5,6,7,9-tetrahydro-benzo[a]heptalen-7-yl]-benzamide



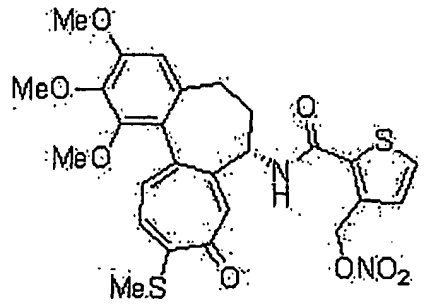
^{1}H NMR(400MHz, $CDCl_3$); δ2.25-2.63(m, 4H), 2.47(s, 3H), 3.74(s, 3H), 3.91(s, 3H), 3.97(S, 3H), 4.85-4.92(m, 1H), 5.27(d, *J*=4.8Hz, 2H), 6.53(d, *J*=8.4Hz, 1H), 6.57(s, 1H), 6.61(s, 1H), 7.20(d, *J*=10.4Hz, 1H), 7.42(d, *J*=10Hz, 1H), 7.63(s, 1H), 7.72(d, *J*=8Hz, 1H), 9.13(d, *J*=6.8Hz, 1H), 12.29(s, 1H)

Example 21: 4-nitrooxymethyl-thiophene-2-carboxylic acid [(7S)-1,2,3-trimethoxy-10-methylsulfanyl-9-oxo-5,6,7,9-tetrahydro-benzo[a]heptalen-7-yl]-amide



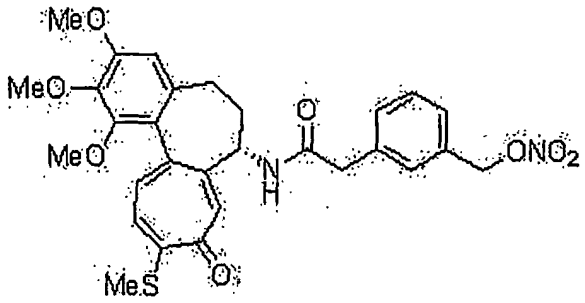
^{1}H NMR(400MHz, $CDCl_3$); δ2.21-2.29(m, 1H), 2.34-2.46(m, 5H), 2.51-2.58(m, 1H), 3.71(s, 3H), 3.91(s, 3H), 3.96(S, 3H), 4.88-4.95(m, 1H), 5.18(dd, *J*=12.8, 20Hz, 2H), 6.56(s, 1H), 7.17(d, *J*=10.4Hz, 1H), 7.30(s, 1H), 7.41(d, *J*=10.4Hz, 1H), 7.61(s, 1H), 7.74(s, 1H), 8.81(d, *J*=6.4Hz, 1H)

Example 22: 3-nitrooxymethyl-thiophene-2-carboxylic acid [(7S)-1,2,3-trimethoxy-10-methylsulfanyl-9-oxo-5,6,7,9-tetrahydro-benzo[a]heptalen-7-yl]-amide



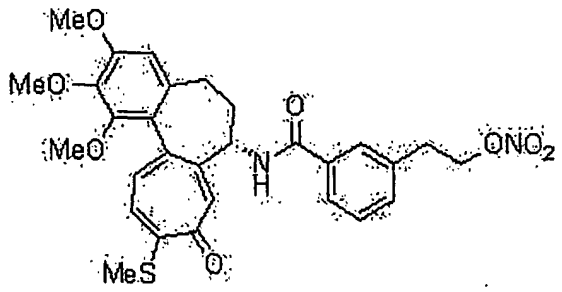
^{1}H NMR(400MHz, $CDCl_3$); δ2.04-2.17(m, 1H), 2.30-2.45(m, 5H), 2.57-2.64(m, 1H), 3.73(s, 3H), 3.91(s, 3H), 3.96(S, 3H), 4.67-4.88(m, 1H), 5.66(d, *J*=13.6Hz, 1H), 5.80(d, *J*=13.6Hz, 1H), 6.56(s, 1H), 7.02(d, *J*=4.4Hz, 1H), 7.08(d, *J*=10.4Hz, 1H), 7.26(d, *J*=4.4Hz, 1H), 7.29(d, *J*=10.4Hz, 1H), 7.43(s, 1H), 7.45(d, *J*=7.2Hz, 1H)

Example 23: 2-(3-nitrooxymethyl-phenyl)-N-[(7S)-1,2,3-trimethoxy-10-methylsulfanyl-9-oxo-5,6,7,9-tetrahydro-benzo[a]heptalen-7-yl]-acetamide



^{1}H NMR(400MHz, $CDCl_3$); δ1.82-1.95(m, 1H), 2.21-2.28(m, 1H), 2.31-2.42(m, 1H), 2.45(s, 3H), 2.47-2.53(m, 1H), 3.51(d, *J*=14.0Hz, 1H), 3.64(s, 3H), 3.66(d, *J*=14.0Hz, 1H), 3.88(s, 3H), 3.93(s, 3H), 4.66-4.72(m, 1H), 5.40(s, 2H), 6.51(s, 1H), 7.11(d, *J*=10.4Hz, 1H), 7.24-7.38(m, 5H), 7.48(s, 1H), 7.90(d, *J*=7.2Hz, 1H)

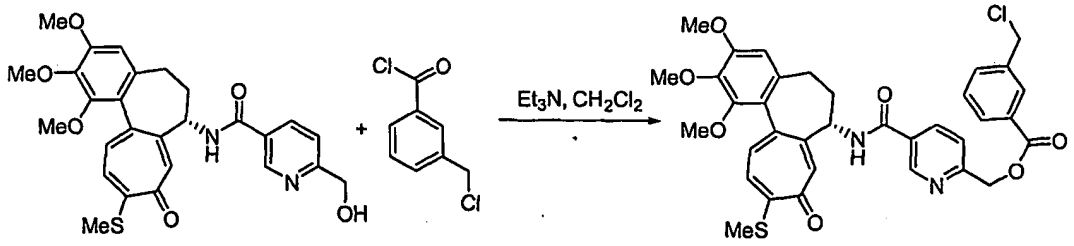
Example 24: 3-(2-nitrooxy-ethyl)-N-[(7S)-1,2,3-trimethoxy-10-methylsulfanyl-9-oxo-5,6,7,9-tetrahydro-benzo[a]heptalen-7-yl]-benzamide



^{1}H NMR(400MHz, $CDCl_3$); δ2.05-2.11(m, 1H), 2.17-2.56(m, 2H), 2.46(s, 3H), 2.56-2.60(m, 1H), 2.81-2.91(m, 2H), 3.69(s, 3H), 3.91(s, 3H), 3.97(s, 3H), 4.45(t, *J*=6.8,Hz, 2H), 4.89-4.95(m, 1H), 6.56(s, 1H), 7.12-7.24(m, 3H), 7.39(d, *J*=10.4Hz, 1H), 7.61-7.64(m, 2H), 7.69(s, 1H), 8.22(d, *J*=6.8Hz, 1H)

Example 25: Preparation of 3-nitrooxy-benzoic acid-5-[(7S)-1,2,3-trimethoxy-10-methylsulfanyl-9-oxo-5,6,7,9-tetrahydro-benzo[a]heptalen-7-yl-carbamoyl]-pyridine-2-yl-methylester

<Step 1> Preparation of 3-chloromethyl-benzoic acid-{5-[(7S)-1,2,3-trimethoxy-10-methylsulfanyl-9-oxo-5,6,7,9-tetrahydro-benzo[a]heptalen-7-yl-carbamonyl]-pyridine-2-yl}-methylester

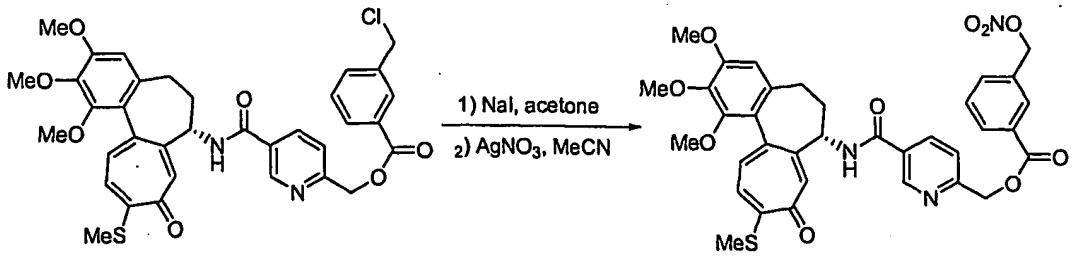


A compound prepared in step 1 of the Example 1 (100 mg, 0.19 mmol) was dissolved in 3 ml of dichloromethane. 3-(Chloromethyl) benzoylchloride (0.030 ml, 0.21 mmol) and triethylamine (0.082 ml, 0.59 mmol) were slowly added therein, and the mixture was reacted at room temperature for 10 minutes. Water was added to quench the reaction, and aqueous layer was

extracted with dichloromethane. Combined organic layer was dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure. The residue was purified by column chromatography (ethyl acetate), to give 99 mg (yield: 79%, yellow solid) of the target compound.

^{1}H NMR (400MHz, $CDCl_3$): δ2.14-2.16(m, 1H), 2.30-2.39(m, 1H), 2.43-2.45(m, 1H), 2.48(s, 3H), 2.68-2.96(m, 1H), 3.67(s, 3H), 3.90(s, 3H), 3.91(s, 3H), 4.83-4.93(m, 1H), 4.88(s, 2H), 5.51(s, 2H), 6.77(s, 1H), 7.27(s, 1H), 7.52(t, J=7.7Hz, 1H), 7.65-7.70(m, 2H), 8.05(d, J=7.7Hz, 1H), 8.14(s, 1H), 8.28(d, J=10.6Hz, 1H), 9.02(s, 1H)

<Step 2> Preparation of 3-nitrooxy-benzoic acid-{5-[(7S)-1,2,3-trimethoxy-10-methylsulfanyl-9-oxo-5,6,7,9-tetrahydro-benzo[a]heptalen-7-yl-carbamoyl]-pyridine-2-yl}-methylester



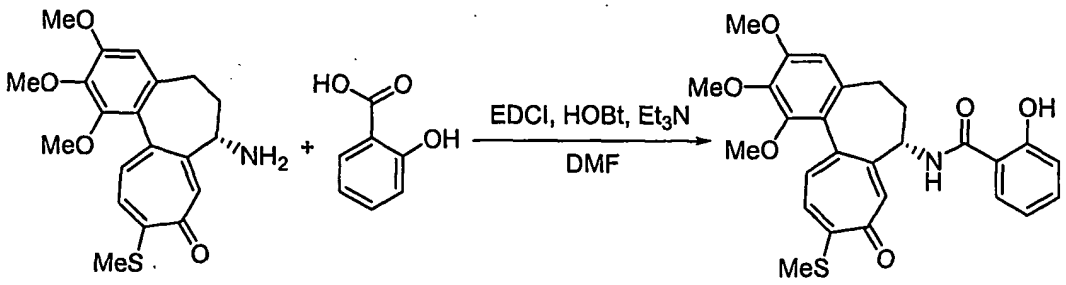
A compound prepared in the step 1 (90 mg, 0.13 mmol) and sodium iodide (31 mg, 0.20 mmol) were dissolved in 3 ml of acetone, and the mixture was reacted at room temperature for 1 day. Water was added to quench the reaction, and aqueous layer was extracted with ethyl acetate. Solvent was concentrated under reduced pressure. The reaction concentrate and silver nitrate (3 0mg, 0.045 mmol) were dissolved in 5 ml of acetonitrile, and the mixture was reacted at room temperature for 1 hour. Water was added to quench the reaction, and aqueous layer was extracted with ethyl acetate. Solvent was concentrated under reduced pressure. The residue was purified by short column chromatography (ethyl acetate) and PLC, to give 26 mg (yield: 29%, yellow solid) of the target compound.

^{1}H NMR (400MHz, $CDCl_3$): δ2.09-2.17(m, 1H), 2.35-2.41(m, 2H), 2.44(s, 3H), 2.55-2.58(m, 1H), 3.74(s, 3H), 3.91(s, 3H), 3.96(s, 3H), 4.92-4.95(m, 1H), 5.45(s, 2H), 5.47(s, 2H), 6.56(s, 1H), 7.13(d, J=10.2Hz, 1H), 7.35(t, J=9.1Hz, 2H), 7.50(t, J=8.5Hz, 1H), 7.54(s, 1H), 7.62(d, J=7.3Hz, 1H), 8.14(d, J=8.3Hz, 2H), 8.24(dd, J=2.2, 6.2Hz, 1H, ArH), 8.36(d, J=6.9Hz, 1H), 9.09(s, 1H)

Example 26~34

Compounds of Example 26 - Example 34 were synthesized in analogy to the procedure as described in Example 25, and intermediates were prepared by the method described as follows.

<Intermediate 16> Preparation of 2-hydroxy-*N*-[(7S)-1,2,3-trimethoxy-10-methylsulfanyl-9-oxo-5,6,7,9-tetrahydro-benzo[a]heptalen-7-yl]-benzamide

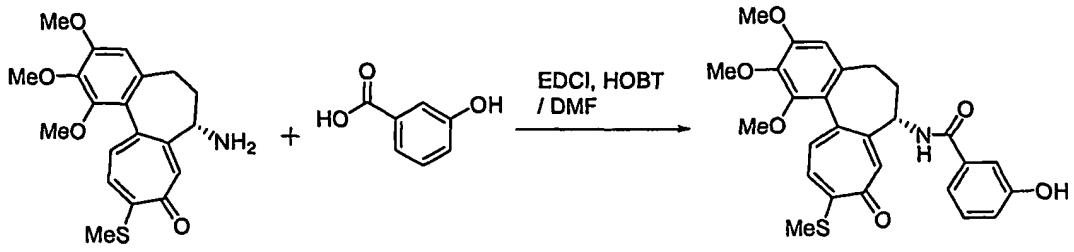


2-Hydroxy-benzoic acid (203 mg, 1.47 mmol), EDCI (385 mg, 2.01 mmol) and HOBt (271 mg, 2.01 mmol) were dissolved in dimethylformamide (10 ml). Triethylamine (0.37 ml, 2.67 mmol) was added therein, and the mixture was stirred at room temperature for 1 day. 7-Amino-1,2,3-trimethoxy-10-methylsulfonyl-6,7-dihydro-5*H*-benzo[a]-heptalen-9-one (500 mg, 1.34 mmol) was added

therein, and the mixture was stirred at room temperature for 1 day. Water was added to quench the reaction, and aqueous layer was extracted with diethyl ether. Combined organic layer was dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure. The residue was purified by column chromatography (hexane:ethyl acetate = 1:5) and recrystallized in methanol, to give 516 mg (yield: 78%, yellow solid) of the target compound.

^{1}H NMR (400MHz, $CDCl_3$): δ2.14-2.17(m, 1H), 2.31-2.37(m, 1H), 2.40-2.44(s, 1H), 2.45(s, 3H), 2.56-2.60(m, 1H), 3.74(s, 3H), 3.91(s, 3H), 3.97(s, 3H), 4.85-4.92(m, 1H), 6.56(s, 1H), 6.64(d, J=7.7Hz, 1H), 6.77(d, J=8.4Hz, 1H), 7.15(d, J=10.6Hz, 1H), 7.22(d, J=7.3Hz, 1H), 7.38(d, J=10.6Hz, 1H), 7.57(s, 1H), 7.71(d, J=8.0Hz, 1H)

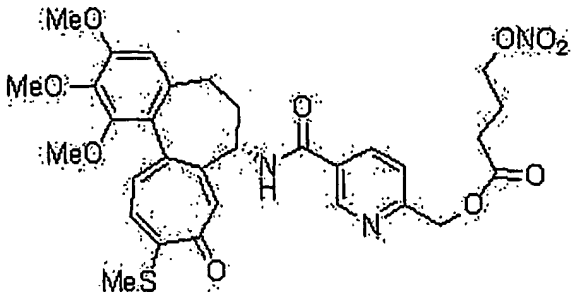
<Intermediate 17> Preparation of 3-hydroxy-*N*-[(7S)-1,2,3-trimethoxy-10-methylsulfanyl-9-oxo-5,6,7,9-tetrahydro-benzo[a]heptalen-7-yl]-benzamide



According to the similar procedure in the preparation method of intermediate 16, by using 3-hydroxybenzoicacid (497 mg, 1.33 mmol), 558 mg (yield: 85%, yellow solid) of the target compound was obtained.

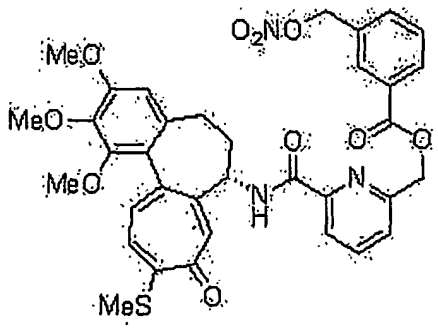
^{1}H NMR (400MHz, DMSO-d_6): δ2.07-2.15(m, 1H), 2.18-2.35(m, 6H), 3.55(s, 3H), 3.80(s, 3H), 3.84(s, 3H), 4.53-4.56(m, 1H), 6.81(s, 1H), 6.92(d, *J*=6.8Hz, 1H), 7.10(s, 1H), 7.15-7.31(m, 5H), 8.98(d, *J*=7.6Hz, 1H, -NH), 9.70(s, 1H, -OH)

Example 26: 4-nitrooxybutyric acid-5-[(7S)-1,2,3-trimethoxy-10-methylsulfanyl-9-oxo-5,6,7,9-tetrahydro-benzo[a]heptalen-7-yl-carbamoyl]-pyridine-2-yl-methylester



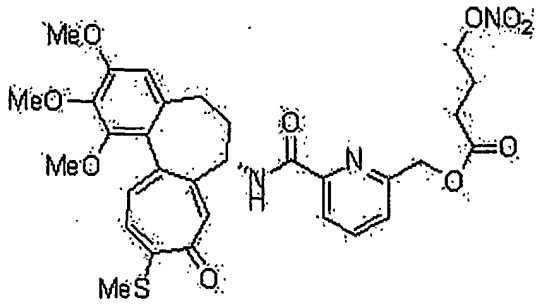
^{1}H NMR (400MHz, $CDCl_3$): δ2.06-2.16(m, 1H), 2.08-2.11(m, 2H), 2.31-2.49(m, 1H), 2.45(s, 3H), 2.57(t, J=7.1Hz, 2H), 3.75(s, 3H), 3.91(s, 3H), 3.96(s, 3H), 4.52(t, J=6.4Hz, 2H), 4.91-4.94(m, 1H), 5.21(s, 2H), 6.56(s, 1H), 7.13(d, J=10.9Hz, 1H), 7.21-7.25(m, 1H), 7.38(d, J=10.6Hz, 1H), 7.52(s, 1H), 8.22(d, J=8.0Hz, 1H), 9.05(s, 1H)

Example 27: 3-nitrooxymethyl-benzoicacid-6-[(7S)-1,2,3-trimethoxy-10-methylsulfanyl-9-oxo-5,6,7,9-tetrahydro-benzo[a]heptalen-7-yl-carbamoyl]-pyridine-2-yl-methylester



^{1}H NMR (400MHz, $CDCl_3$): δ1.84-1.92(m, 1H), 2.33-2.60(m, 1H), 2.43(s, 3H), 2.46-2.54(m, 1H) 2.55-2.60(m, 1H), 3.73(s, 3H), 3.92(s, 3H), 3.96(S, 3H), 4.74-4.81(m, 1H), 5.51(s, 2H) 5.57(d, *J*=4.0Hz, 1H), 6.56(s, 1H), 7.04(d, *J*=10.4Hz, 1H), 7.25(d, *J*=10.4Hz, 1H), 7.54-7.60(m, 2H), 7.65(d, *J*=7.6Hz, 1H), 7.87(t, *J*=8.0Hz, 1H), 7.99(d, *J*=7.6Hz, 1H), 8.21(s, 2H), 8.22(s, 1H), 8.41(d, *J*=7.2Hz, 1H)

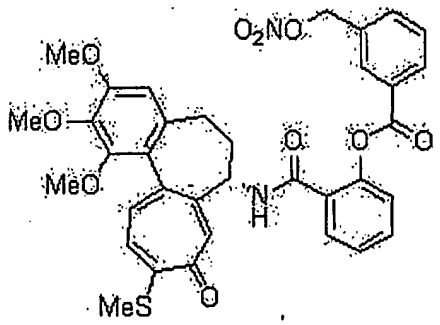
Example 28: 4-nitrooxybutyric acid-6-[(7S)-1,2,3-trimethoxy-10-methylsulfanyl-9-oxo-5,6,7,9-tetrahydro-benzo[a]heptalen-7-yl-carbamoyl]-pyridine-2-yl-methylester



^{1}H NMR (400MHz, $CDCl_3$): δ2.02-2.09(m, 1H), 2.11-2.18(m, 2H), 2.21-2.40(m, 2H), 2.43(s, 3H), 2.47-2.56(m, 1H), 2.63(t, *J*=7.2Hz, 2H), 3.73(s, 3H), 3.92(s, 3H),

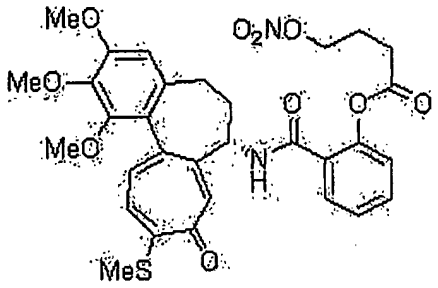
3.96(S, 3H), 4.57(t, J=6.4Hz, 2H), 4.77-4.83(m, 1H), 5.30(d, J=4.8Hz, 2H), 6.58(s, 1H), 7.05(d, J=10.4Hz, 1H), 7.25(S, 1H), 7.32(d, J=10.4Hz, 1H), 7.52(d, J=8.0Hz, 1H), 7.84(t, J=8.0Hz, 1H), 7.98(d, J=8.0Hz, 1H), 8.45(d, J=6.8Hz, 1H)

Example 29: 3-nitrooxymethyl-benzoic acid-2-[(7S)-1,2,3-trimethoxy-10-methylsulfanyl-9-oxo-5,6,7,9-tetrahydro-benzo[a]heptalen-7-yl-carbamoyl]-phenylester



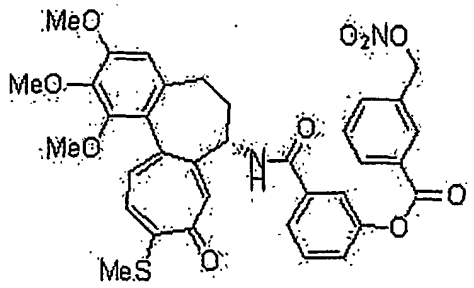
^{1}H NMR (400MHz, $CDCl_3$): δ1.48-1.55(m, 1H), 1.83-1.89(m, 1H), 2.22-2.39(m, 2H), 2.42(s, 3H), 3.62(s, 3H), 3.87(s, 3H), 3.92(s, 3H), 4.63-4.70(m, 1H), 5.54(s, 2H), 6.47(s, 1H), 6.89(d, J=7.4Hz, 1H), 7.03(d, J=10.2Hz, 1H), 7.17(s, 1H), 7.23(d, J=10.1Hz, 1H), 7.35(t, J=7.3Hz, 1H), 7.54(t, J=7.4Hz, 1H), 7.58(t, J=7.6Hz, 1H), 7.72(d, J=8.0Hz, 1H), 7.83(d, J=7.7Hz, 1H), 8.26(m, 2H)

Example 30: 4-nitrooxybutyric acid-2-[(7S)-1,2,3-trimethoxy-10-methylsulfanyl-9-oxo-5,6,7,9-tetrahydro-benzo[a]heptalen-7-yl-carbamoyl]-phenylester



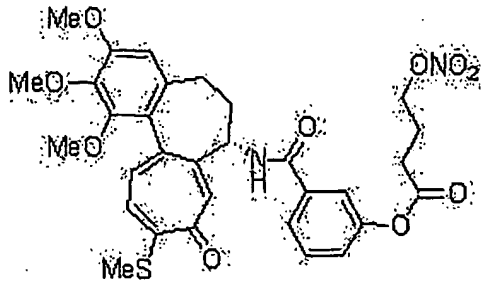
^{1}H NMR (400MHz, $CDCl_3$): δ1.60-1.90(m, 1H), 2.10-2.14(m, 2H), 2.26-2.39(m, 1H), 2.44(s, 3H), 2.46-2.51(m, 1H), 2.56-2.61(m, 1H), 2.80(t, J=7.1Hz, 2H), 3.70(s, 3H), 3.92(s, 3H), 3.96(s, 3H), 4.55(t, J=6.2Hz, 2H), 4.74-4.81(m, 1H), 6.57(s, 1H), 6.82(d, J=6.9Hz, 1H), 7.08(d, J=10.6Hz, 1H), 7.13(d, J=9.1Hz, 1H), 7.23(s, 1H), 7.28-7.36(m, 1H), 7.47(t, J=9.9Hz, 1H), 7.68(d, J=9.5Hz, 1H)

Example 31: 3-nitrooxymethyl-benzoic acid-3-[(7S)-1,2,3-trimethoxy-10-methylsulfanyl-9-oxo-5,6,7,9-tetrahydro-benzo[a]heptalen-7-yl-carbamoyl]-phenylester



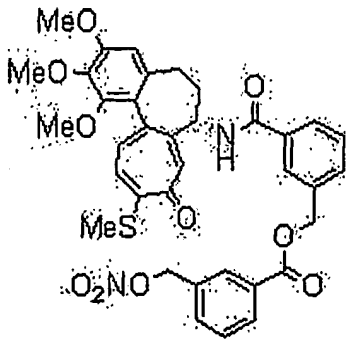
^{1}H NMR (400MHz, $CDCl_3$): δ2.05-2.15(m, 1H), 2.23-2.59(m, 6H), 3.75(s, 3H), 3.91(s, 3H), 3.97(s, 3H), 4.90-5.00(m, 1H), 5.49(s, 2H), 6.56(s, 1H), 7.03(d, *J*=10.4Hz, 1H), 7.27(s, 2H), 7.34(d, *J*=8.8Hz, 2H) 7.53-7.65(m, 2H), 7.76(s, 1H), 7.77(d, *J*=6.4Hz, 1H), 8.13(m, 3H)

Example 32: 4-nitrooxybutyric acid-3-[(7S)-1,2,3-trimethoxy-10-methylsulfanyl-9-oxo-5,6,7,9-tetrahydro-benzo[a]heptalen-7-yl-carbamoyl]-phenylester



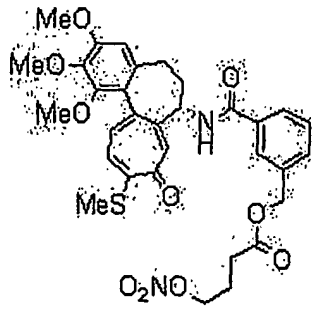
^{1}H NMR (400MHz, $CDCl_3$): δ2.04-2.18(m, 3H), 2.25-2.60(m, 6H), 2.65(t, *J*=6.8Hz, 2H), 3.74(s, 3H), 3.91(s, 3H), 3.96(s, 3H), 4.56(t, *J*=6.4Hz, 2H), 4.87-4.94(m, 1H), 6.56(s, 1H), 7.08-7.12(m, 2H), 7.26-7.35(m, 2H), 7.48(s, 1H), 7.59(s, 1H), 7.71(d, *J*=8.0Hz, 1H), 7.75 (d, *J*=7.6Hz, 1H, -NH)

Example 33: 3-nitrooxymethyl-benzoic acid-3-[(7S)-1,2,3-trimethoxy-10-methyl-sulfanyl-9-oxo-5,6,7,9-tetrahydro-benzo[a]heptalen-7-yl-carbamoyl]-benzylester



^{1}H NMR (400MHz, $CDCl_3$): δ2.05-2.18(m, 1H), 2.31-2.47(m, 2H), 2.40(s, 3H), 2.55-2.59(m, 1H), 3.75(s, 3H), 3.91(s, 3H), 3.97(s, 3H), 4.90-4.96(m, 1H), 5.24(s, 2H), 5.44(s, 2H), 6.56(s, 1H), 7.09(d, *J*=10.6Hz, 1H), 7.27(dd, *J*=7.6, 7.6Hz, 1H), 7.36(d, *J*=10.6Hz, 1H), 7.44(d, *J*=7.6Hz, 1H), 7.45(dd, *J*=8.0, 7.6Hz, 1H), 7.58(d, *J*=8.0Hz, 1H), 7.59(s, 1H), 7.79(d, *J*=7.6Hz, 1H), 7.91(s, 1H), 8.05(d, *J*=7.6Hz, 1H), 8.06(s, 1H), 8.18(d, *J*=7.2Hz, 1H)

Example 34: 4-nitrooxybutyric acid-3-[(7S)-1,2,3-trimethoxy-10-methylsulfanyl-9-oxo-5,6,7,9-tetrahydro-benzo[a]heptalen-7-yl-carbamoyl]-benzylester

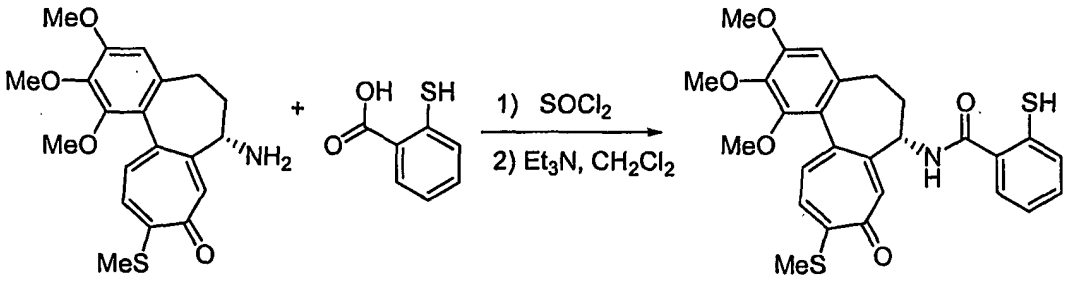


^{1}H NMR (400MHz, $CDCl_3$): δ2.00-2.14(m, 3H), 2.31-2.51(m, 4H), 2.44(s, 3H), 2.56-2.60(m, 1H), 3.75(s, 3H), 3.92(s, 3H), 3.97(s, 3H), 4.49(t, *J*=6.2Hz, 2H), 4.89-4.95(m, 1H), 4.99(d, *J*=12.4Hz, 1H), 5.04(d, *J*=12.4Hz,

1H), 6.56(s, 1H), 7.11(d, J=10.6Hz, 1H), 7.26(dd, J=7.6, 7.6Hz, 1H), 7.35-7.37(m, 2H), 7.54(s, 1H), 7.76(d, J=8.0Hz, 1H), 7.81(s, 1H), 8.00(d, J=7.6Hz, 1H)

Example 35: Preparation of 2-nitrosothio-N-[(7S)-1,2,3-trimethoxy-10-methylsulfanyl-9-oxo-5,6,7,9-tetrahydro-benzo[a]heptalen-7-yl]-benzamide

<Step 1> Preparation of 2-mercapto-*N*-[(7S)-1,2,3-trimethoxy-10-methylsulfanyl-9-oxo-5,6,7,9-tetrahydro-benzo[a]heptalen-7-yl]-benzamide

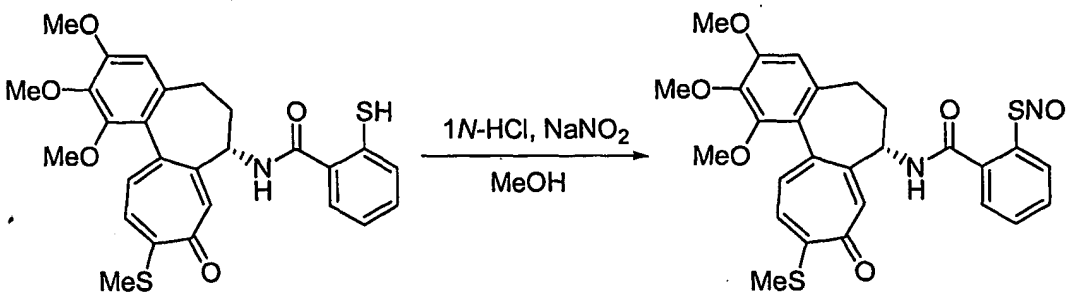


Excess thionylchloride was added in thiosalycilic acid (115 mg, 0.75 mmol), and the mixture was stirred with heating for 1 day. The reaction mixture was concentrated under reduced pressure to remove thionylchloride, and to give a chloride compound. 7-

amino-1,2,3-trimethoxy-10-methylsulfonyl-6,7-dihydro-5*H*-benzo[a]-heptalen-9-one (243 mg, 0.62 mmol) was dissolved in purified dichloromethane. Triethylamine (0.26 ml, 1.86 mmol) was slowly added therein. Thiosalycilic chloride dissolved in dichloromethane was also added therein at 0℃, and the mixture was stirred for 30 minutes. Water was added to quench the reaction, and aqueous layer was extracted with chloroform. Combined organic layer was dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure. The residue was purified by column chromatography (chloroform:methanol = 12:1), to give 210 mg (yield: 66%, white solid) of the target compound.

^{1}H NMR (400MHz, $CDCl_3$) : δ2.06-2.17(m, 1H), 2.33-2.49(m, 2H), 2.42(s, 3H), 2.54-2.59(m, 1H), 3.72(s, 3H), 3.92(s, 3H), 3.96(s, 3H), 4.86-4.93(m, 1H), 6.56(s, 1H), 7.06(d, J=10.6Hz, 1H), 7.14(t, J=7.5Hz, 1H), 7.24(d, J=7.7Hz, 1H), 7.31(d, J=10.6Hz, 1H), 7.51(s, 1H), 7.58(d, J=7.3Hz, 1H), 7.63(t, J=8.6Hz, 2H)

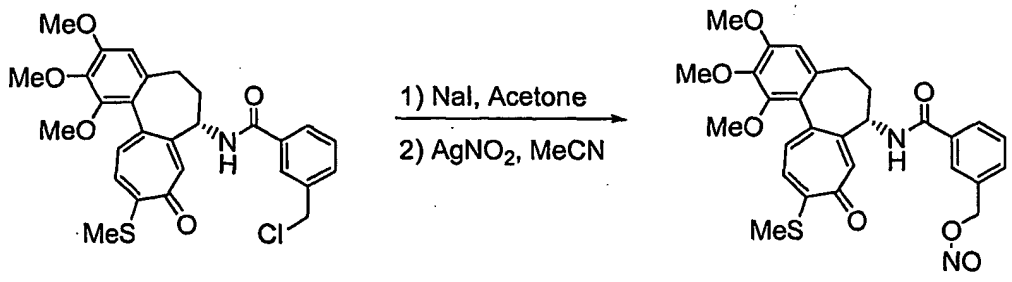
<Step 2> Preparation of 2-nitrosothio-N-[(7S)-1,2,3-trimethoxy-10-methylsulfanyl-9-oxo-5,6,7,9-tetrahydro-benzo[a]heptalen-7-yl]-benzamide



Compound prepared in step 1 (40 mg, 0.078 mmol) was dissolved in methanol. 1 N HCl aqueous solution (3 ml) was added therein. Sodium nitrite ($NaNO_2$, 6.5 mg, 0.094 mmol) dissolved in water (1.5 ml) was also added therein, and the mixture was stirred at room temperature for 1 hour. Sodium hydrogen carbonate was added to quench the reaction, and aqueous layer was extracted with chloroform. Combined organic layer was dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure. The residue was purified by column chromatography (chloroform:methanol = 12:1) and recrystallized in methanol, to give 34.1 mg (yield: 81%, yellow solid) of the target compound.

^{1}H NMR (400MHz, $CDCl_3$): δ2.07-2.14(m, 1H), 2.31-2.37(m, 1H), 2.42(s, 3H), 2.45-2.48(m, 1H), 2.54-2.59(m, 1H), 3.72(s, 3H), 3.91(s, 3H), 3.96(s, 3H), 4.88-4.94(m, 1H), 6.56(s, 1H), 7.06(d, J=10.6Hz, 1H), 7.10(t, J=7.5Hz, 1H), 7.21(t, J=6.9Hz, 1H), 7.31(d, J=10.6Hz, 1H), 7.54(s, 1H), 7.64(t, J=8.6Hz, 2H), 7.72(d, J=7.3Hz, 1H)

Example 36: Preparation of 3-nitrosooxymethyl-*N*-[(7S)-1,2,3-trimethoxy-10-methylsulfanyl-9-oxo-5,6,7,9-tetrahydro-benzo[a]heptalen-7-yl]-benzamide



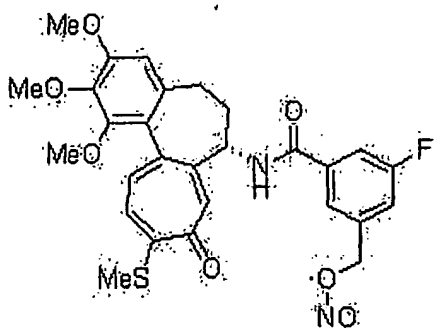
3-Chloromethyl-*N*-[(7S)-1,2,3-trimethoxy-10-methylsulfanyl-9-oxo-5,6,7,9-tetrahydrobenzo[a]heptalen-7-yl]-benzamide (119.8 mg, 0.228 mmol) and sodium iodide (136,5 mg, 0.911 mmol) were dissolved in acetone (15 ml), and the mixture was stirred at 55℃ for 1 day. The reaction mixture was extracted with chloroform and

washed with saturated sodium chloride aqueous solution. Combined organic layer was dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure. The residue and silver nitrite (125.5 mg, 0.812 mmol) were dissolved in acetonitrile (5 ml), and the mixture was stirred at room temperature for 1 day. The reaction mixture was extracted with chloroform. Combined organic layer was dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure. The residue was purified by column chromatography (ethyl acetate:chloroform = 4:1), to give 35.4 mg (yield: 32.4%, yellow solid) of the target compound.

^{1}H NMR (400 MHz, $CDCl_3$): δ2.35-2.55(m, 3H), 2.48(s, 3H), 2.62-2.66(m, 1H), 3.76(s, 3H), 3.84(d, *J*=12.8Hz, 1H), 3.92(s, 3H), 3.98(s, 3H), 4.26(d, *J*=12.8Hz, 1H), 4.91-4.97(m, 1H), 6.58(s, 1H), 6.96(dd, *J*=7.7, 7.7Hz, 1H), 7.22(d, *J*=10.6Hz, 1H), 7.26-7.30(m, 2H), 7.44-7.47(m, 2H), 7.86(s, 1H), 9.30(d, *J*=6.8Hz, 1H)

Example 37: 3-fluoro-5-nitrosooxymethyl-*N*-[(7S)-1,2,3-trimethoxy-10-methylsulfanyl-9-oxo-5,6,7,9-tetrahydro-benzo[a]heptalen-7-yl]-benzamide

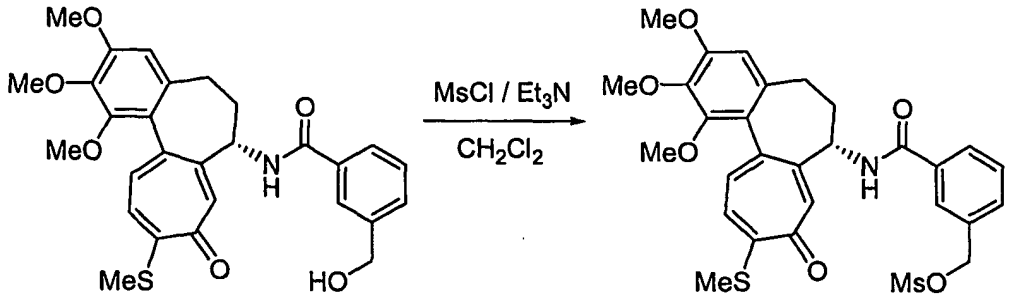
A target compound was synthesized in analogy to the procedure as described in the Example 36.



^{1}H NMR (400 MHz, $CDCl_3$): δ2.04~2.20(m, 1H), 2.47(s, 3H), 2.52~2.62(m, 2H), 2.67~2.72(m, 1H), 3.81(s, 3H), 3.91(s, 3H), 3.97(s, 3H), 4.71(s, 2H), 4.93~4.96(m, 1H), 6.42(d, J=6.2, 1H), 6.58(s, 1H), 7.14~7.21(m, 2H), 7.30(d, J=8.4, 1H), 7.42(s, 1H), 7.53(d, J=10.6, 1H)

Example 38: Preparation of 3-nitrosothiomethyl-*N*-[(7S)-1,2,3-trimethoxy-10-methylsulfanyl-9-oxo-5,6,7,9-tetrahydro-benzo[a]heptalen-7-yl]-benzamide

<Step 1> Preparation of methanesulfonic acid-3-[(7S)-1,2,3-trimethoxy-10-methylsulfanyl-9-oxo-5,6,7,9-tetrahydro-benzo[a]heptalen-7-yl-carbamoyl]-benzyl ester

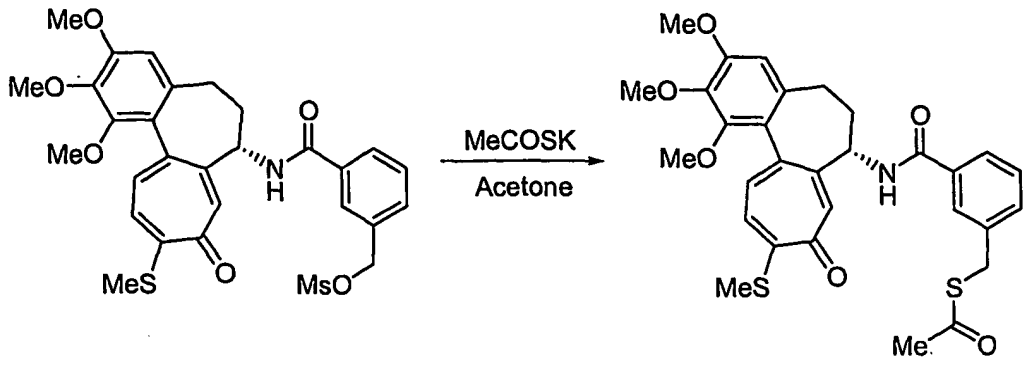


3-Hydroxymethyl-*N*-[(7S)-1,2,3-trimethoxy-10-methylsulfanyl-9-oxo-5,6,7,9-tetrahydro-benzo[a]heptalen-7-yl]-benzamide (252.5 mg, 0.497 mmol) was dissolved in dichloromethane (10 ml), and the temperature was lowered into 0℃. Methanesulfonylchloride (42.4 ㎕, 0.547 mmol) and triethylamine (0.104 ml, 0.745 mmol) were added therein, and the mixture was stirred at room temperature for 2

hours. The reaction mixture was extracted with chloroform. Combined organic layer was dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure. The residue was purified by column chromatography (ethyl acetate:chloroform = 3:2), to give 182.3 mg (yield: 62.6%, yellow solid) of the target compound.

^{1}H NMR (400 MHz, $CDCl_3$): δ2.14-2.21(m, 1H), 2.31-2.50(m, 2H), 2.46(s, 3H), 2.57-2.62(m, 1H), 2.85(s, 3H), 3.76(s, 3H), 3.92(s, 3H), 3.97(s, 3H), 4.87-4.94(m, 1H), 5.06(d, *J*=12.7Hz, 1H), 5.10(d, *J*=12.7Hz, 1H), 6.57(s, 1H), 7.14(d, *J*=10.6Hz, 1H), 7.28(dd, *J*=7.7, 7.7Hz, 1H), 7.38-7.42(m, 2H), 7.56(s, 1H), 7.74(d, *J*=8.0Hz, 1H), 8.24(d, *J*=7.7Hz, 1H)

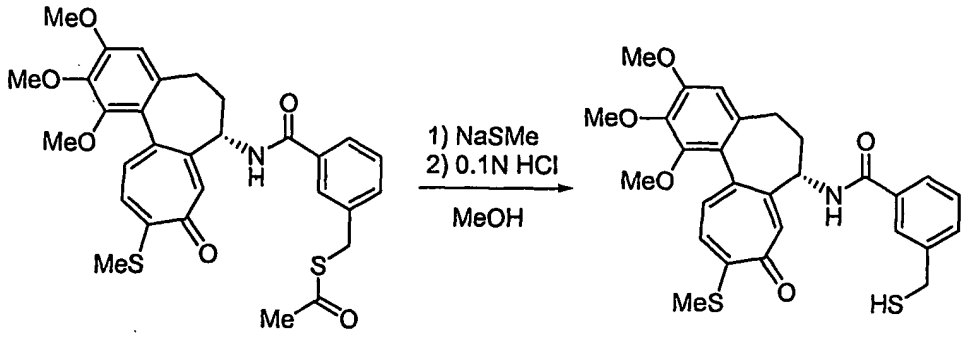
<Step 2> Preparation of thioacetic acid-*S*-3-[(7S)-1,2,3-trimethoxy-10-methylsulfanyl-9-oxo-5,6,7,9-tetrahydro-benzo[a]heptalen-7-yl-carbamoyl]-benzyl ester



A compound prepared in the step 1 (182.3 mg, 0.311 mol) was dissolved in acetone (6 ml), and the temperature was lowered into 0℃. Potassium thioacetate (53.2 mg, 0.467 mmol) was slowly added therein at 0℃, and the mixture was stirred for 1 hour. The reaction mixture was extracted with chloroform. Combined organic layer was dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure, to give 173.6 mg (yield: 98.6%, yellow solid) of the target compound.

^{1}H NMR (400MHz, $CDCl_3$): δ2.05-2.14(m, 1H), 2.30(s, 2H), 2.32-2.49(m, 2H), 2.44(s, 3H), 2.52-2.61(m, 1H), 3.74(s, 3H), 3.92(s, 3H), 3.97(s, 3H), 4.01(d, *J*=13.9Hz, 1H), 4.05(d, *J*=13.9Hz, 1H), 4.87-4.93(m, 1H), 6.56(s, 1H), 7.10(d, *J*=10.3Hz, 1H), 7.21(dd, *J*=7.7, 7.7Hz, 1H), 7.32-7.36(m, 2H), 7.49(s, 1H), 7.64-7.71(m, 3H)

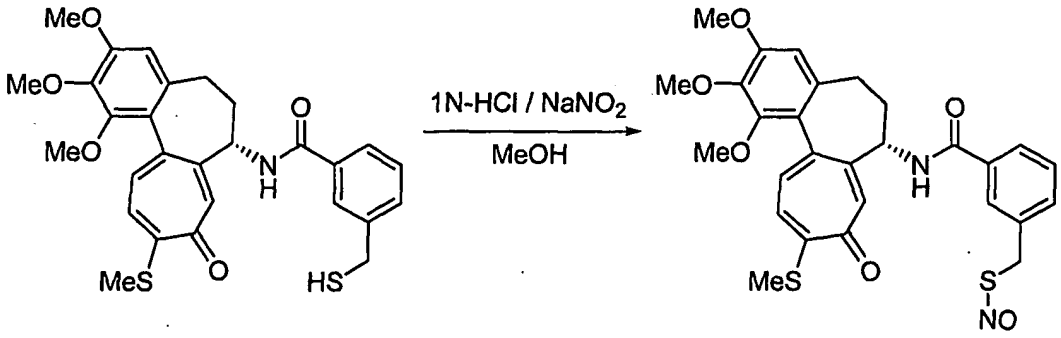
<Step 3> Preparation of 3-mercaptomethyl-*N*-[(7S)-1,2,3-trimethoxy-10-methylsulfanyl-9-oxo-5,6,7,9-tetrahydro-benzo[a]heptalen-7-yl]-benzamide



A compound prepared in the step 2 (165.2 mg, 0.292 mol) was dissolved in methanol (6 ml), and the temperature was lowered into 0℃. Sodium thiomethoxide (21.5 mg, 0.307 mmol) was slowly added therein at 0℃, and the mixture was stirred at room temperature for 30 minutes. Reaction was quenched by adding 0.1N HCl aqueous solution. The reaction mixture was extracted with chloroform and washed with saturated sodium chloride aqueous solution. Combined organic layer was dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure. The residue was purified by column chromatography (ethyl acetate:chloroform = 1:1), to give 182.3 mg (yield: 62.6%, yellow solid) of the target compound.

^{1}H NMR (400MHz, $CDCl_3$): δ1.70(t, *J*=7.3Hz, 1H), 2.12-2.19(m, 1H), 2.31-2.50(m, 2H), 2.18(s, 3H), 2.57-2.61(m, 1H), 3.58(d, *J*=7.3Hz, 1H), 3.74(s, 3H), 3.92(s, 3H), 3.97(s, 3H), 4.89-4.95(m, 1H), 6.57(s, 1H), 7.12(d, *J*=10.3Hz, 1H), 7.19(dd, *J*=7.7, 7.7Hz, 1H), 7.34(d, *J*=7.7Hz, 1H), 7.37(d, *J*=10.3Hz, 1H), 7.55(s, 1H), 7.59(d, *J*=7.7Hz, 1H), 7.70(s, 1H), 7.93(d, *J*=7.3Hz, 1H)

<Step 4> Preparation of 3-nitrosothiomethyl-*N*-[(7S)-1,2,3-trimethoxy-10-methylsulfanyl-9-oxo-5,6,7,9-tetrahydro-benzo[a]heptalen-7-yl]-benzamide



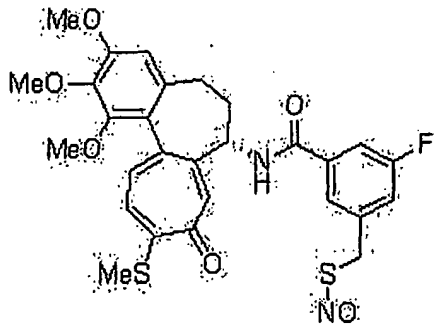
A compound prepared in the step 3 (101.2 mg, 0.193 mol) was dissolved in methanol (3 ml) and dimethylformamide (3ml), and the temperature was lowered into 0℃. 0.1N HCl aqueous solution (3 ml) and

sodium nitrite (16.0 mg) were slowly added therein at 0℃, and the mixture was stirred at room temperature for 2 hours. The reaction mixture was extracted with chloroform and washed with saturated sodium carbonate solution. Combined organic layer was dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure. The residue was purified by column chromatography (ethyl acetate:chloroform:hexane = 3:2:1.5), to give 19.5 mg (yield: 18.3%, yellow solid) of the target compound.

^{1}H NMR (400MHz, $CDCl_3$): δ2.08-2.16(m, 1H), 2.31-2.51(m, 2H), 2.45(s, 3H), 2.57-2.61(m, 1H), 3.57(s, 3H), 3.80(s, 2H), 3.92(s, 3H), 3.97(s, 3H), 4.89-4.96(m, 1H), 6.57(s, 1H), 7.10(d, *J*=10.3Hz, 1H), 7.25(dd, *J*=7.7, 7.7Hz, 1H), 7.36(d, *J*=10.3Hz, 1H), 7.38(d, *J*=7.7Hz, 1H), 7.53(s, 1H), 7.68(d, *J*=7.7Hz, 1H), 7.77(s, 1H), 7.79(d, *J*=7.0Hz, 1H)

Example 39: 3-fluoro-5-nitrosothiomethyl-*N*-[(7S)-1,2,3-trimethoxy-10-methylsulfanyl-9-oxo-5,6,7,9-tetrahydro-benzo[a]heptalen-7-yl]-benzamide

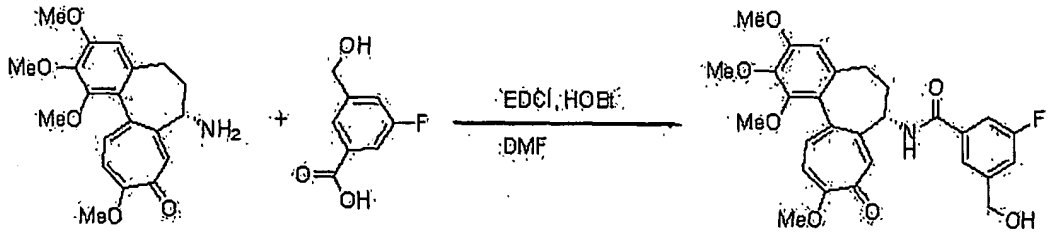
A target compound of Example 39 was synthesized in analogy to the procedure as described in the Example 38.



^{1}H NMR (400MHz, $CDCl_3$): δ2.30~2.48(m, 3H), 2.44(s, 3H), 2.56~2.59(m, 1H), 3.57(q, J=13.9, 16.5, 2H), 3.73(s, 3H), 3.91(s, 3H), 3.97(s, 3H), 4.97~5.03(m, 1H), 6.57(s, 1H), 7.02(d, J=8.4, 1H), 7.15(d, J=10.6, 1H), 7.39(d, J=10.2, 1H), 7.43(s, 1H), 7.64(d, J=9.1, 1H), 7.73(s, 1H), 8.96(d, J=7.3, 1H)

Example 40: Preparation of 3-fluoro-5-nitrooxymethyl-N-[(7S)-1,2,3,10-tetramethoxy-9-oxo-5,6,7,9-tetrahydro-benzo[a]heptalen-7-yl]-benzamide

<Step 1> Preparation of 3-fluoro-5-hydroxymethyl-N-[(7S)-1,2,3,10-tetramethoxy-9-oxo-5,6,7,9-tetrahydro-benzo[a]heptalen-7-yl]-benzamide

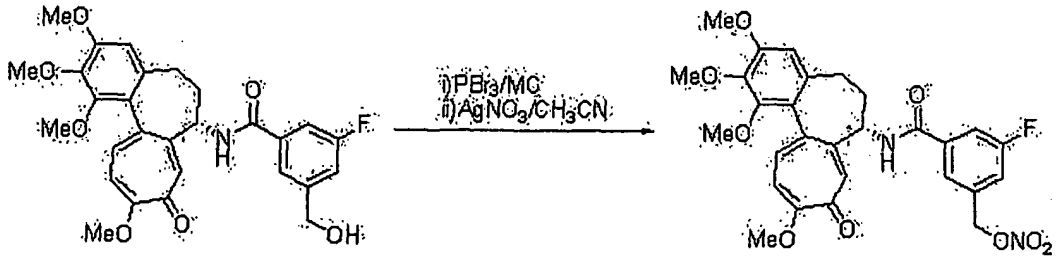


Deacetylcolchicine (150 mg, 0.42 mmol), 3-fluoro-5-hydroxymethyl-benzoic acid (85 mg, 0.50 mmol) and HOBt (67 mg, 0.50 mmol) were dissolved in dimethylformamide solution (2 ml), and the temperature was lowered into 0℃. EDCI (95 mg, 0.50 mmol) was slowly added therein at 0℃, and the mixture was stirred at room temperature. Water was added to quench the reaction, and aqueous layer was extracted with ethyl acetate. Combined organic layer was dried over anhydrous sodium sulfate, filtered and concentrated

under reduced pressure. The residue was purified by column chromatography (chloroform:ethyl acetate = 2:1), to give 110 mg (yield: 52%, yellow solid) of the target compound.

^{1}H NMR (400MHz, $CDCl_3$): δ2.40-2.58(m, 3H), 2.62-2.69(m, 1H), 3.64-3.78(m, 1H), 3.74(s, 3H), 3.92(s, 3H), 3.98(s, 3H), 4.07(S, 3H), 4.23-4.28(m, 1H), 4.81-4.88(m, 1H), 6.58(s, 1H), 6.87(d, *J*=9.2Hz, 1H), 6.97(d, *J*=9.2Hz, 1H), 7.03(d, *J*=10.4Hz, 1H), 7.38(s, 1H), 7.50(d, *J*=10.4Hz, 1H), 7.96(s, 1H), 9.64(d, *J*=6.0Hz, 1H)

<Step 2> Preparation of 3-fluoro-5-nitrooxymethyl-N-[(7S)-1,2,3,10-tetramethoxy-9-oxo-5,6,7,9-tetrahydro-benzo[a]heptalen-7-yl]-benzamide



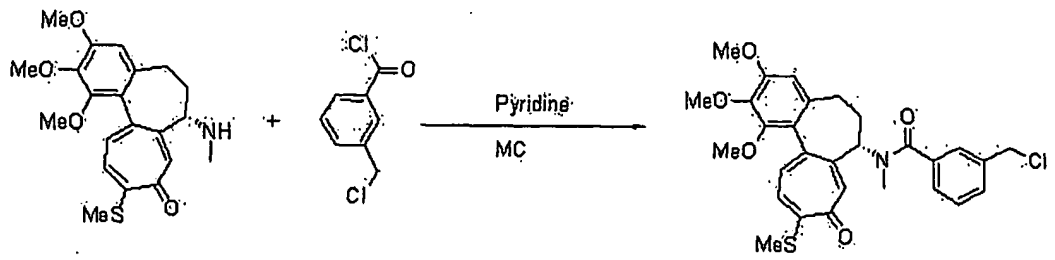
According to the similar procedure in the step 2 and 3 of Example 5, by using a compound prepared in the

step 1 (90 mg, 0.18 mmol), 30 mg (yield: 30%, yellow solid) of the target compound was obtained.

^{1}H NMR (400MHz, $CDCl_3$): δ2.38-2.49(m, 3H), 2.58-2.59(m, 1H), 3.75(s, 3H), 3.92(s, 3H), 3.98(s, 3H), 4.05(S, 3H), 4.89-4.93(m, 1H), 4.94(d, *J*=12.8Hz, 1H), 5.10(d, *J*=12.8Hz, 1H), 6.58(s, 1H), 6.96(d, *J*=8.4Hz, 1H), 7.01(d, *J*=10.4Hz, 1H), 7.17(d, *J*=8.4Hz, 1H), 7.45(s, 1H), 7.48(d, *J*=10.4Hz, 1H), 7.89(s, 1H), 9.25(d, *J*=6.4Hz, 1H)

Example 41: Preparation of 3-nitrooxymethyl-N-methyl-N-[(7S)-1,2,3-trimethoxy-10-methylsulfanyl-9-oxo-5,6,7,9-tetrahydro-benzo[a]heptalen-7-yl]-benzamide

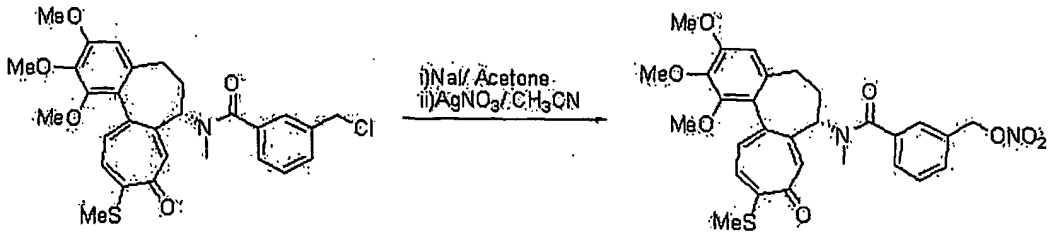
<Step 1> Preparation of 3-chloromethyl-N-methyl-N-[(7S)-1,2,3-trimethoxy-10-methylsulfanyl-9-oxo-5,6,7,9-tetrahydro-benzo[a]heptalen-7-yl]-benzamide



Thiodemecolcine (50 mg, 0.129 mmol) and pyridine (0.012 ml, 0.154 mmol) were dissolved in dichloromethane, and the temperature was lowered into 0℃. 3-(Chloromethyl)benzoyl chloride (0.022 ml, 0.154 mmol) was slowly added therein at 0℃, and the mixture was stirred at room temperature. Water was added to quench the reaction, and aqueous layer was extracted with ethyl acetate. Combined organic layer was dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure. The residue was purified by column chromatography (chloroform:ethyl acetate = 2:1), to give 45 mg (yield: 67%, yellow solid) of the target compound.

^{1}H NMR (400MHz, $CDCl_3$): δ2.24-2.38(m, 2H), 2.44(s, 3H),2.45-2.58(m, 1H), 2.61-2.74(m, 1H), 3.25(s, 3H), 3.72(s, 3H), 3.91(s, 3H), 3.92(s, 3H), 4.54(s, 2H), 5.05(br, 1H), 6.57(s, 1H), 7.06(d, *J*=10.0Hz, 1H), 7.09(s, 1H), 7.22-7.41(m, 5H)

<Step 2> Preparation of 3-nitrooxymethyl-N-methyl-N-[(7S)-1,2,3-trimethoxy-10-methylsulfanyl-9-oxo-5,6,7,9-tetrahydro-benzo[a]heptalen-7-yl]-benzamide



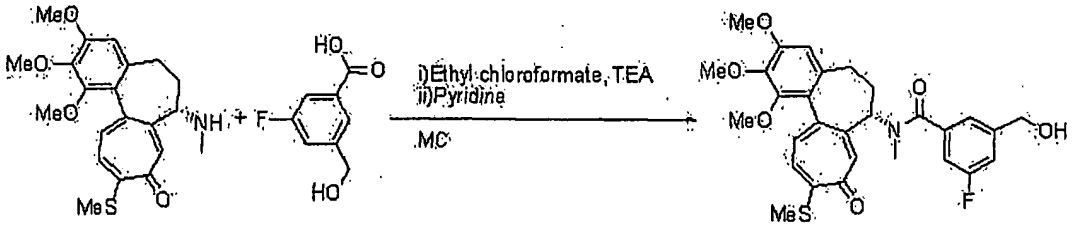
According to the similar procedure in the step 2 of Example 25, by using a compound prepared in the step 1 (45 mg, 0.085 mmol), 40 mg (yield: 83%, yellow solid) of the target compound was obtained.

^{1}H NMR (400MHz, $CDCl_3$): δ2.24-2.38(m, 2H), 2.44(s, 3H),2.45-2.58(m, 1H), 2.61-2.74(m, 1H), 3.24(s, 3H), 3.71(s, 3H), 3.91(s, 3H), 3.92(s, 3H), 4.77(br, 1H), 5.58(s, 2H), 6.85(s, 2H), 7.21(d, *J*=10.0Hz, 1H), 7.29(d, *J*=10.4Hz, 1H), 7.40-7.53(m, 4H)

Example 42: Preparation of 3-fluoro-N-methyl-5-nitrooxymethyl-N-[(7S)-1,2,3-trimethoxy-10-

methylsulfanyl-9-oxo-5,6,7,9-tetrahydro-benzo[a]heptalen-7-yl]-benzamide

<Step 1> Preparation of 3-fluoro-5-hydroxymethyl-N-methyl-N-[(7S)-1,2,3-trimethoxy-10-methylsulfanyl-9-oxo-5,6,7,9-tetrahydro-benzo[a]heptalen-7-yl]-benzamide

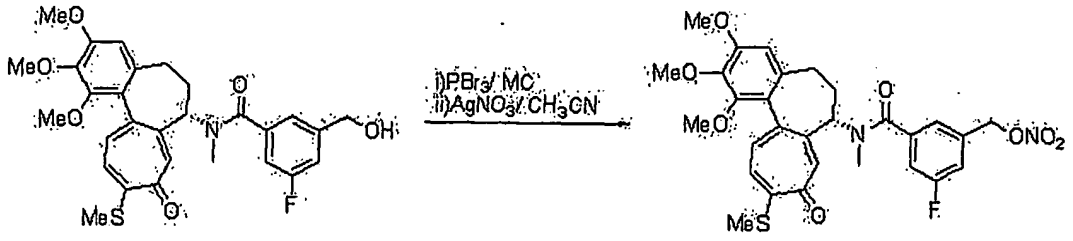


3-Fluoro-5-hydroxymethyl-benzoic acid (52 mg, 0.309 mmol) was dissolved in dichloromethane (3 ml) under a nitrogen atmosphere. Ethylchloroformate (0.022 ml, 0.231 mmol) and TEA (0.042 ml, 0.309 mmol) were slowly added therein at 0℃, and the mixture was stirred at 0℃ for 30 minutes. Pyridine (0.012 ml, 0.154 mmol) and thiodemecolcine (60 mg, 0.154 mmol) were also added therein, and the mixture was stirred at room temperature for 3 hours. Water was added to quench the reaction, and aqueous layer was extracted with ethyl acetate. Combined organic layer was dried over anhydrous sodium sulfate, filtered and

concentrated under reduced pressure. The residue was purified by column chromatography (chloroform:ethyl acetate = 2:1), to give 30 mg (yield: 21%, yellow solid) of the target compound.

^{1}H NMR (400MHz, $CDCl_3$): δ1.76(br, 1H), 2.24-2.38(m, 2H), 2.44(s, 3H),2.45-2.58(m, 1H), 2.61-2.74(m, 1H), 3.23(s, 3H), 3.72(s, 3H), 3.93(s, 3H), 3.97(s, 3H), 4.65(s, 2H), 5.02(br, 1H), 6.57(s, 1H), 6.92(s, 1H), 6.99-7.24(m, 4H), 7.34(s, 1H)

<Step 2> Preparation of 3-fluoro-N-methyl-5-nitrooxymethyl-N-[(7S)-1,2,3-trimethoxy-10-methylsulfanyl-9-oxo-5,6,7,9-tetrahydro-benzo[a]heptalen-7-yl]-benzamide



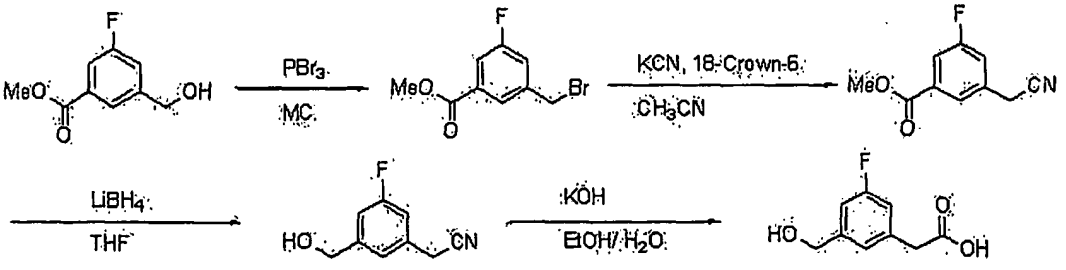
According to the similar procedure in the step 2 and 3 of Example 5, by using a compound prepared in the

step 1 (55 mg, 0.101 mmol), 20 mg (yield: 37%, yellow solid) of the target compound was obtained.

^{1}H NMR (400MHz, $CDCl_3$): δ2.22-2.41(m, 2H), 2.45(s, 3H), 2.45-2.58(m, 1H), 2.61-2.74(m, 1H), 3.24(s, 3H), 3.75(s, 3H), 3.91(s, 3H), 3.95(s, 3H), 5.02(br, 1H), 5.39(s, 2H), 6.57(s, 1H), 7.05-7.24(m, 5H), 7.34(s, 1H)

Example 43: Preparation of 2-(3-fluoro-5-nitrooxymethyl-phenyl)-N-[(7S)-1,2,3-trimethoxy-10-methylsulfanyl-9-oxo-5,6,7,9-tetrahydro-benzo[a]heptalen-7-yl]-acetamide

<Step 1> Preparation of (3-fluoro-5-hydroxymethyl-phenyl)-acetic acid

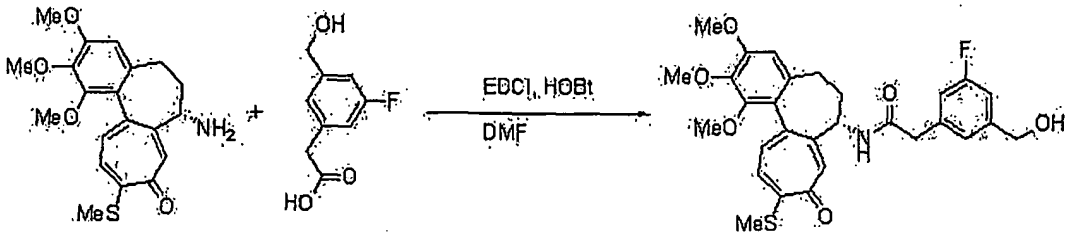


A compound prepared in the step 1 of intermediate 6 (2.5 g, 13.57 mmol) was dissolved in dichloromethane

(30 ml). PBr_3 (1.15 ml, 12.21 mmol) was slowly added therein, and the mixture was stirred at room temperature for 3 hours to give 3-bromomethyl-5-fluoro-benzoic acid methyl ester (1.6 g, yield: 50%, white solid). 3-Bromomethyl-5-fluoro-benzoic acid methyl ester (1.5 g, 6.1 mmol), KCN (1.6 g, 24.4 mmol) and 18-crown-6 (820 mg, 3.1 mmol) were dissolved in acetonitrile (10 ml). The reaction mixture was stirred at room temperature for 18 hours to give 3-cyanomethyl-5-fluoro-benzoic acid methyl ester (900 mg, yield: 77%, white solid). 3-Cyanomethyl-5-fluoro-benzoic acid methyl ester (900 mg, 4.65 mmol) was dissolved in tetrahydrofuran (10ml). 2M Lithiumborohydride tetrahydrofuran (2.3 ml, 4.6 mmol) was slowly added therein, and the mixture was refluxed, to give (3-fluoro-5-hydroxymethyl-phenyl)-acetonitrile (430 mg, yield: 56%, white solid). (3-Fluoro-5-hydroxymethyl-phenyl)-acetonitrile (400 mg, 2.42 mmol) and potassiumhydroxide (1.34 g, 23.8 mmol) were dissolved in ethanol (10 ml) and water (5 ml). The reaction mixture was refluxed for 24 hours to give 356 mg (yield: 80%, white solid) of the target compound.

1H NMR(400MHz, CD_3OD); δ3.62(s, 2H), 4.59(s, 2H), 6.94(d, *J*=9.6Hz, 1H), 7.04(d, *J*=9.6Hz, 1H), 7.08(s, 1H)

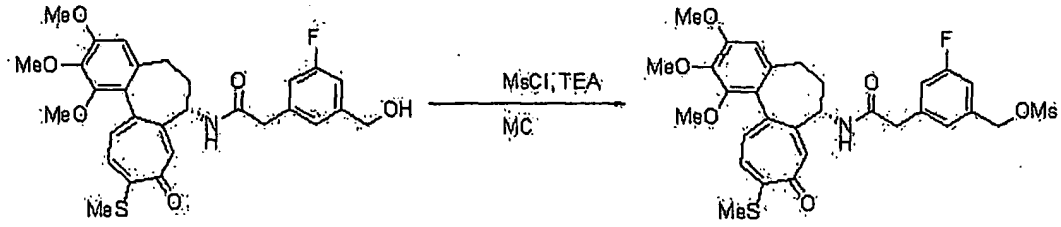
<Step 2> Preparation of 2-(3-fluoro-5-hydroxymethyl-phenyl)-N-[(7S)-1,2,3-trimethoxy-10-methylsulfanyl-9-oxo-5,6,7,9-tetrahydro-benzo[a]heptalen-7-yl]-acetamide



According to the similar procedure in the step 1 of Example 40, by using a compound prepared in the step 1 (30 mg, 0.16 mmol), 58 mg (yield: 70%, yellow solid) of the target compound was obtained.

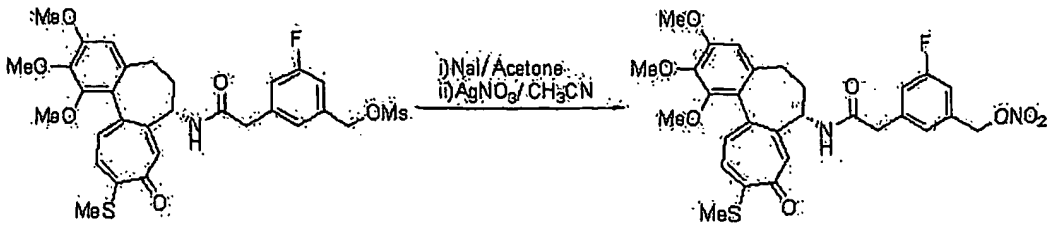
^{1}H NMR (400MHz, $CDCl_3$): δ1.92-2.01(m, 1H), 2.19-2.40(m, 2H), 2.41(s, 3H), 2.46-2.52(m, 1H), 3.46(d, *J*=14.0,Hz, 1H), 3.52(d, *J*=14.0,Hz, 1H), 3.63(s, 3H), 3.89(s, 3H), 3.91(s, 3H), 4.64-4.74(m, 1H), 4.75(s, 2H), 6.53(s, 1H), 6.86-6.94(m, 3H), 7.07(d, *J*=10.8Hz, 1H), 7.13(s, 1H), 7.30(d, *J*=10.8Hz, 1H), 7.39(s, 1H)

<Step 3> Preparation of methanesulfonic acid 3-fluoro-5-[(7S)-(1,2,3-trimethoxy-10-methylsulfanyl-9-oxo-5,6,7,9-tetrahydro-benzo[a]heptalen-7-ylcarbamoyl)-methyl]-benzyl ester



According to the similar procedure in the step 1 of Example 38, by using a compound prepared in the step 2 (58 mg, 0.107 mmol), 60 mg (yield: 90%, yellow solid) of the target compound was obtained.

<Step 4> Preparation of 2-(3-fluoro-5-nitrooxymethyl-phenyl)-N-[(7S)-1,2,3-trimethoxy-10-methylsulfanyl-9-oxo-5,6,7,9-tetrahydro-benzo[a]heptalen-7-yl]-acetamide



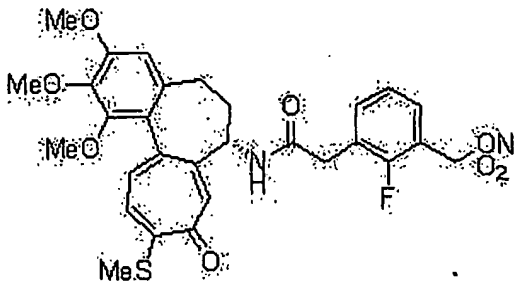
According to the similar procedure in the step 2 of Example 25, by using a compound prepared in the step 3 (60 mg, 0.097 mmol), 30 mg (yield: 83%, yellow solid) of the target compound was obtained.

^{1}H NMR (400MHz, $CDCl_3$): δ1.92-1.98(m, 1H), 2.22-2.42(m, 2H), 2.46(s, 3H), 2.49-2.52(m, 1H), 3.50(d, *J*=14.0,Hz, 1H), 3.65(s, 3H), 3.66(d, *J*=14.0,Hz, 1H), 3.89(s, 3H), 3.93(s, 3H), 4.64-4.74(m, 1H), 5.37(s, 2H), 6.52(s, 1H), 6.97(d, *J*=8.8Hz, 1H), 7.11-7.17(m, 3H), 7.35(d, *J*=10.8Hz, 1H), 7.50(s, 1H), 8.04(d, *J*=7.2Hz, 1H)

Example 44 : 2-(2-fluoro-5-nitrooxymethyl-phenyl)-N-[(7S)-1,2,3-trimethoxy-10-methylsulfanyl-9-oxo-5,6,7,9-tetrahydro-benzo[a]heptalen-7-yl]-acetamide

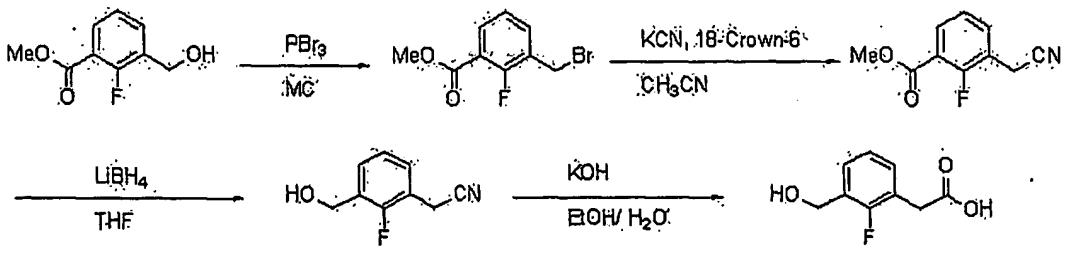
A compound of Example 44 was synthesized in analogy to the procedure as described in Example 43,

and an intermediate was prepared by the method described as follows.



^{1}H NMR (400MHz, $CDCl_3$): δ1.89-1.93(m, 1H), 2.26-2.29(m, 1H), 2.34-2.40(m, 1H), 2.43(s, 3H), 2.49-2.54(m, 1H), 3.61(s, 3H), 3.62(d, *J*=15.2Hz, 1H), 3.72(d, *J*=15.2Hz, 1H), 3.89(s, 3H), 3.92(s, 3H), 4.70-4.75(m, 1H), 5.49(s, 2H), 6.53(s, 1H), 7.06-7.11(m, 2H), 7.25-7.31(m, 2H), 7.35(t, *J*=7.6Hz, 1H), 7.44(s, 1H), 7.57(d, *J*=7.2Hz, 1H)

<Intermediate 18> Preparation of (2-fluoro-5-hydroxymethyl-phenyl)-acetic acid



According to the similar procedure in the step 1 of Example 43, by using a compound prepared in the intermediate 5 (2.2 g, 11.94 mmol), 400 mg (yield: 90%, white solid) of the target compound was obtained.

Experimental Example 1: Cytotoxicity test to cancer cell lines

Cytotoxicity to A549 (Korea Research Institute of Chemical Technology), SK-OV-3 (Korea Research Institute of Chemical Technology), SK-MEL-2 (Korea Research Institute of Chemical Technology), HCT-15 (Korea Research Institute of Chemical Technology) and MCF7 (Korean Cell Line Bank, Seoul National University School of medicine) cells was measured by Sulforhodamin-B (SRB) method (1989, National Cancer Institute (NCI)) which was developed for the measurement of *in vitro* anticancer activity of a drug. Cells were separated using 0.25% trypsin-EDTA solution, leading to the preparation of a cell suspension by 5×10^3 ~ 2×10^4 cells/well. Then, the suspension was distributed into a 96 well plate by 100 ㎕/well, which was cultured in a 37℃, 5% CO_2 incubator for 24 hours. Compounds prepared in examples of the present invention were used as a sample. Precisely, the compound was

dissolved in dimethylsulfoxide and diluted with RPMI 1640 medium before being used as a sample. The final concentration of the sample used varied ranging 1 μM ~ 0.00001 μM. The medium was removed from the 96 well plate and then diluted sample solution was added by 100 ㎕/well, followed by further culture in a 37℃, 5% CO_2 incubator for 48 hours. Tz (time zero) plate was collected from the point of adding the sample. Upon completing the culture, the medium was removed from each well along with Tz plate, then 10% trichloroacetic acid (TCA) was added by 100 ㎕/well. The plate was left at 4℃ for 1 hour to let cells be fixed on the floor of the plate. After cells were fixed completely, the plate was washed with water 5-6 times to remove the remaining trichloroacetic acid solution completely and moisture was completely dried at room temperature. 0.4% Sulforhodamine-B was dissolved in 1% acetic acid solution to prepare a staining solution. Cells were stained for 30 minutes with a dye added by 100 ㎕ to each well of the completely dried plate. Then, the plate was washed with 1% acetic acid solution 5-6 times to remove sulforhodamine-B remained uncombined with cells. Then, the plate was dried at room temperature. 10 mM Tris solution was added by 100 ㎕/well thereto to dissolve

the dye, and optical density (OD) was measured at 520 nm with a micro plate reader.

ED_{50} (concentration that inhibits cancer cell growth 50%, 50% effective dose, nM/ml) of the sample to cancer cell was calculated as follows. OD value at the point of beginning the culture with the sample was determined as Tz (time zero) value. OD value of a well to be cultured without the sample was determined as a control value (C). OD value of a well pretreated with the sample was determined as experimental value (T). After calculating Tz, C and T, cytotoxicity of the sample was measured by the below <Mathematical Formula 1>.

<Mathematical Formula 1>

$T \leq Tz,\ (T-Tz)/(C-Tz) \times 100$

$T > Tz,\ (T-Tz)/Tz \times 100$

ED_{50}, the concentration that can inhibit cancer cell growth by 50%, was calculated by using a regression analysis of lotus program based on the degree of cytotoxicity obtained by the <Mathematical Formula 1>.

Each ED_{50} of paclitaxel, doxorubicin and colchicine, which were used as controls, was also calculated by the same way as described in the above.

Results were presented in Table 1.

<Table 1>

Cytotoxicity to cancer cell lines (NT: Not tested)

Example	Cytotoxicity [ED_{50} : nM]				
	A549	SK-OV-3	SK-MEL-2	HCT15	MCF-7
Paclitaxel	0.3	1.2	0.1	0.1	0.9
Doxorubicin	13.0	47.0	16.0	25.0	50.0
Colchicine	21.0	18.0	6.0	9.0	NT
Example 1	1.8	1.0	0.4	0.6	NT
Example 2	15.8	9.3	5.4	2.3	2.9
Example 3	2.4	4.0	1.0	4.8	2.3
Example 4	0.01	0.01	0.01	0.03	0.01
Example 5	1.0	1.0	3.1	4.4	NT
Example 6	0.70	0.37	0.08	0.27	0.16
Example 7	>50	>50	>50	>50	>50
Example 8	0.02	0.04	0.01	0.03	0.01
Example 9	0.49	0.34	0.22	0.64	0.13
Example 10	0.28	0.23	0.12	0.39	0.09
Example 11	>50	>50	>50	>50	39.0
Example 12	0.05	0.27	0.11	0.05	0.03
Example 13	0.13	0.18	0.04	0.11	0.02
Example 14	28.2	30.6	32.8	9.9	11.2
Example 15	30.2	>50	22.5	23.4	18.8

Example 16	1.6	1.0	1.0	1.0	NT
Example 17	0.21	0.19	0.17	0.19	0.08
Example 18	8.1	3.8	1.0	5.3	NT
Example 19	23.5	34.8	31.0	10.1	8.2
Example 20	3.93	1.83	2.47	1.03	1.15
Example 21	0.97	0.59	0.29	0.13	0.10
Example 22	1.11	1.41	0.87	0.95	0.21
Example 23	5.4	5.6	3.5	3.4	3.3
Example 25	8.8	5.4	14.0	15.0	NT
Example 26	21.0	>50.0	47.0	>50.0	NT
Example 27	5.0	16.0	7.0	2.0	NT
Example 28	6.0	15.0	4.0	3.0	NT
Example 29	1.1	3.0	0.9	0.8	NT
Example 30	3.6	8.2	3.6	5.2	NT
Example 31	1.10	3.0	0.9	0.8	NT
Example 32	6.2	1.0	0.05	0.08	NT
Example 33	3.8	6.6	2.9	1.0	NT
Example 34	0.06	0.01	0.01	0.01	0.01
Example 35	>50	>50	41.9	20.9	24.7
Example 36	2.9	1.1	0.1	0.7	NT
Example 37	>50	38.8	9.4	7.5	10.3
Example 38	2.4	4.1	3.3	3.0	NT
Example 39	40.7	28.2	>50	9.9	5.0
Example 40	43.9	45.6	60.7	24.3	10.5
Example 41	>50	>50	>50	36.4	47.3
Example 42	>50	>50	>50	>50	>50

As shown in Table 1, tricyclic derivatives according to the present invention showed very strong cytotoxicity to cancer cell lines.

Experimental Example 2: Inhibiting effect of tricyclic derivatives of the present invention on tumor growth

In order to investigate inhibiting effect of tricyclic derivatives of the invention on tumor growth, following experiments were performed.

Samples for the experiment were stored in a refrigerator all the time. Different dosages of compounds were administered to a test animal; dosage of a compound prepared in the Example 8 was 10 mg/kg, dosage of a compound prepared in the Example 12 was 1, 3, 10 mg/kg respectively and dosage of a positive control was 2.5 mg/kg (Taxol) and 2 mg/kg (Adriamycin). The compound of the Example 12 was dissolved in 4% tween 80, and the positive control Taxol was dissolved in a mixed solvent of 5% ethanol + 25% cremophor + 75% PBS. The prepared sample is subject to be precipitated, so that tip sonication was performed right before the administration to disperse it well.

7 week-old female S.P.F. BALB/c nude mice, provided by Charles River Co., Japan, were used as test

animals. The test animals were adapted in a Hepa-filter room over a week before the test. The temperature was 21±2℃, humidity was 55±5% and 12-hour light and dark cycle was automatically repeated in that lab. Solid feed (CheilJedang) was sterilized by radioactive rays and drinking water was also sterilized by autoclave. Feed and water were taken by the animal freely. Cancer cell line used in this experiment was NCI-H460 (human lung tumor cell line) provided by Korea Research Institute of Bioscience and Biotechnology.

The tumor cell line, stored in liquid nitrogen, was thawed and cultured in a 37℃, 5% CO_2 incubator for a required time. Upon completing the culture, all the cells were recovered and cell concentration of the culture fluid was adjusted using PBS to 3×10^7 cells/ml. The adjusted cell culture solution was injected hypodermically into armpit between right shoulder girdle and chest wall by 0.3 ml per mouse. From the next day of grafting, NCI-H460 xenografted nude mice were administered intraperitoneal everyday with the sample solution by 0.2 ml/20 g of weight, once a day.

After the grafting of tumor cells, the volume of a tumor in each individual was measured in three dimensions by using a vernier caliper, which was represented in the below <Mathematical Formula 2>.

<Mathematical Formula 2>

Tumor volume = (lengthxwidthxheight)/2

Body weight changes of animal were measured three times a week. Each xenografted nude mouse was sacrificed to separate a tumor, which was then weighed.

All the test results of experimental groups were compared with those of control groups by t-Test to see if there is any significant difference between the two groups.

Changes of the volume and the weight of a tumor were shown in Table 2 and in FIG. 1, 3, and 5, and changes of the body weight of a mouse were shown in Table 3, in FIG. 2 and in FIG. 4.

<Table 2>

Experimental Group	Dosage (mg/kg)	Tumor volume (㎣)						Tumor Weight (mg)
		Day 0	Day 4	Day 7	Day 9	Day 11	Day 14	Day 14
V.C-1	0	0.0± 0.0	28.3± 8.1	53.5± 13.7	98.3± 25.5	167.5± 43.8	295.2± 62.2	1171.2 ±383.5
V.C-2	0	0.0± 0.0	26.7± 3.8	49.6± 12.3	80.9± 26.7	138.2± 54.9	289.0± 53.1	1087.9 ±300.5
Example 8	10	0.0± 0.0	18.0± 3.7*	25.2± 12.8**	29.6± 13.8***	35.2± 14.0***	44.5± 15.0***	143.4± 57.8***
Example 12	1	0.0± 0.0	20.7± 3.1	49.2± 12.2	78.1± 13.0	146.5± 8.8	299.3± 41.5	950.9± 174.8
	3	0.0± 0.0	16.9± 5.1*	31.2± 11.7*	49.7± 15.8**	96.1± 26.1**	206.8± 48.0*	714.5± 103.8*

	10	0.0± 0.0	16.1± 6.7*	24.6± 9.8**	35.2± 12.9***	69.4± 26.9***	103.0±0	390.9± 0
Taxol	2.5	0.0± 0.0	17.5± 4.6***	32.7± 3.8**	47.2±0	-	-	-
Adriamycin	2	0.0± 0.0	21.8± 8.3	33.7± 8.7*	47.6± 16.2**	81.6± 24.5**	138.1± 39.9***	534.1± 87.9

※ Significance test (t-Test) : *(p<0.05), **(p<0.01), ***(p<0.001),

V.C-1 : 4% tween 80,

V.C-2 : 5% ethanol + 25% cremophor + 75% PBS

<Table 3>

Experimental Group	Dosage (mg/kg)	Animal Number (n)	Body weight Change (%)					
			Day 1	Day 3	Day 7	Day 9	Day 11	Day 14
V.C-1	0	6	100.0± 0.0	105.4± 2.5	108.7 ± 2.4	107.2± 3.7	107.8± 4.6	107.8± 3.2
V.C-2	0	6	100.0± 0.0	106.9± 2.4	110.5± 1.5	108.5± 2.6	109.0± 52.6	108.9± 3.6
Example 8	10	5	100.0± 0.0	103.5± 2.2	99.8± 4.3**	92.3± 4.1***	89.3± 3.7***	89.4± 5.3***
Example 12	1	6	100.0± 0.0	105.0± 1.4	104.2± 5.3	99.6± 4.4**	97.5± 3.6**	98.3± 3.2***
	3	6	100.0± 0.0	102.8± 2.0	101.8± 4.2**	99.3± 3.3*	99.1± 2.8**	94.5± 3.9***
	10	6 (Day 14-2)	100.0± 0.0	103.9± 0.9	92.2± 3.5***	89.4± 2.0**	92.3± 2.9***	93.4±0
Taxol	2.5	6 (Day 9-2, Day 11, Day 14-1)	100.0± 0.0	106.0± 1.5	99.2± 4.0***	97.1±0	-	-
Adriamycin	2	6	100.0± 0.0	105.0± 4.1	103.9± 5.5	100.5± 4.6*	96.4± 3.5***	85.1± 1.8***

※ Significance test (t-Test) : *(p<0.05), **(p<0.01), ***(p<0.001),

V.C-1 : 4% tween80,

V.C-2 : 5% ethanol + 25% cremophor + 75% PBS

As shown in Table 2, and FIG. 1, 3 and 5, the size and the weight of a tumor of NCI-H460 xenografted BALB/c nude mouse were remarkably decreased when it was administered with tricyclic derivatives of the present invention (prepared in Example 8 and Example 12), comparing to when being administered with a solvent only (V.C-1, V.C-2) or with a positive control (taxol, adriamycin). In particular, in the case of the compound of the Example 12, the volume and the weight of a tumor were much decreased with dosage of 10 mg/kg than with the dosage of 1 mg/kg. And in the case of the compound of the Example 8, inhibition rate of the tumor volume showed 85% when it was administered with 10 mg/kg. Therefore, decreasing rate of the volume and the weight of a tumor can appreciate in proportion to dosage of tricyclic derivatives of the present invention.

As shown in Table 3 and in FIG. 2 and 4, the weight of NCI-H460 xenografted BALB/c nude mouse was decreased about 10% when it was administered with tricyclic of the present invention (prepared in Example 8 and Example 12), comparing to when being administered

with a solvent only (V.C-1, V.C-2) or with a positive control (taxol, adriamycin).

Therefore, tricyclic derivatives of the present invention make the volume and the weight of a tumor smaller and lighter dose-dependently, and also show excellent anticancer effect. So, tricyclic derivatives of the invention can be effectively used as an anticancer agent and as a anti-proliferation agent.

Experimental Example 3: Inhibitory affect of tricyclic derivatives of the present invention on capillary-like tube formation in HUVEC cells (Capillary-like tube formation assay)

Matrigel used in this experiment was a product of BioCoat. Matrigel was thawed in a refrigerator for 24 hours before use. Thawing matrigel, 96 well plate and yellow tip were put on ice. Then, matrigel was distributed into each well of the plate by 40 ㎕. The polymerization of the plate was performed in a 37℃ incubator for 30 minutes. Each well of the plate was inoculated with 180 ㎕ of HUVEC cell solution (2×10^4 cells/㎖) along with 20 ㎕ of the compound of the Example 12 in serum-free media (0.3, 1, 3, 10 and 30 ㎍

/㎖), followed by further culture for 24 hours.

Tube formation was observed under a microscope to investigate inhibition activity of angiogenesis.

Fumagilin and doxorubicin were used as a positive control.

The results were shown in FIG. 6.

As shown in FIG. 6, tricyclic derivatives of the present invention (prepared in the Example 12) had angiogenesis inhibition activity with dosage over 0.3 ㎍/㎖, which was as good effect as that of fumagilin, a positive control, with the dosage of 10 ㎍/㎖. Further, tricyclic derivatives of the invention (prepared in the Example 12) totally inhibited angiogenesis with the dosage over 10 ㎍/㎖.

Thus, tricyclic derivatives of the present invention can be effectively used as an angiogenesis inhibitor.

Experimental Example 4: Acute toxicity test

5-week-old ICR mice having the weight of 25-35 g (SPF, SLC Co. Japan) were used as test animals. A pair of female and male of the mice was given for the test of each compound. Group T2, T3, T4 and T5 were arranged (10 animals per group) for the test of acute

toxicity of the compound prepared in the Example 12. The compound of the Example 12 was dissolved in a solvent [5% DMSO; 20% tween 80; 75% PBS(-)], which was injected into the abdominal cavity of the mouse (dosage was shown in Table 4), followed by observation for 7 days. Control group (T1) was administered with only a solvent without the compound of the Example 12. And the results were shown in Table 4.

For comparison, colchicine was injected in the test animals by the same method as described in the above. The group treated with colchicine was composed of 6 mice. The test results were shown in Table 5.

Taxol produced by Bristol Myers Sqibb Co. was also used for comparison, and the injection and the test of acute toxicity were performed by the same method as described in the above. The results were shown in Table 6.

<Table 4>

Acute toxicity test with a compound prepared in Example 12

Group	Animal Number	Dosage (mg/kg)	Test Day (Death)							
			1	2	3	4	5	6	7	Total

T1	10	Solvent	-	-	-	-	-	-	-	0/10
T2	10	30	-	-	-	-	-	-	-	0/10
T3	10	40	-	-	-	-	-	-	-	0/10
T4	10	50	-	-	-	-	-	-	-	0/10
T5	10	60	-	-	-	-	-	-	-	0/10

<Table 5>

Acute toxicity test with colchicine

Group	Animal Number	Dosage (mg/kg)	Test Day (Death)							
			1	2	3	4	5	6	7	Total
T1	6	Solvent	-	-	-	-	-	-	-	0/6
T2	6	0.5	-	-	-	-	-	-	-	0/6
T3	6	1.0	-	-	-	-	-	-	-	0/6
T4	6	2.0	-	-	1	-	-	2	-	3/6
T5	5	5.0	-	3	-	1	1	-	-	5/5

<Table 6>

Acute toxicity test with Taxol

Group	Animal Number	Dosage (mg/kg)	Test Day (Death)							
			1	2	3	4	5	6	7	Total
T1	5	4.0	-	-	-	-	-	-	-	0/5
T2	5	6.0	-	1	-	-	-	-	-	1/5
T3	5	9.0	-	-	-	-	2	-	-	2/5
T4	5	13.0	2	2	-	-	-	-	-	4/5
T5	5	20.0	5	-	-	-	-	-	-	5/5

As shown in Table 4, 5 and 6, toxicity of natural colchicine to a mouse was confirmed to be LD_{50} = 2mg/kg, which was very similar to earlier reported value of LD_{50} = 1.6mg/kg [Medicinal Research Reviews, Vol.8, No.1, 77-94 (1988)]. Toxicity of taxol injection, paclitaxel, was LD_{50} = 9 - 13 mg/kg (i.v. administration). But toxicity of the compound of the Example 12 of the present invention was LD_{50} = 60mg/kg, which was 30 times as week toxicity as that of colchicine and also weaker than that of taxol injection. Thus, the compound of the present invention was proved to have less toxicity to normal cells than colchicine or Taxol injection.

INDUSTRIAL APPLICABILITY

Tricyclic derivatives of the present invention have very strong cytotoxicity to cancer cell lines but less toxicity to animals themselves than colchicine or Taxol injection has. Tricyclic derivatives of the invention further decrease the volume and the weight of a tumor and inhibit angiogenesis in HUVEC cells excellently. Therefore, the derivatives can be effectively used as an anticancer agent, anti-proliferation agent and an angiogenesis inhibitor as

well. In addition, tricyclic derivatives of the present invention can be obtained with ease and be formulated easily for oral administration or for injection owing to its water-solubility.